STUDY OF STROMAL EXPRESSION OF CD 10 IN BREAST CARCINOMA AND ITS ROLE AS A PROGNOSTIC MARKER

Dissertation submitted in

partial fulfillment of the regulations required for the award of

M.D. DEGREE

In

PATHOLOGY – BRANCH III

THE TAMILNADU

DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI-TAMILNADU.

APRIL 2017

CERTIFICATE

This is to certify that the dissertation entitled "**STUDY OF STROMAL EXPRESSION OF CD 10 IN BREAST CARCINOMA AND ITS ROLE AS A PROGNOSTIC MARKER"** is a record of bonafide work done by **Dr.Subashini S** in the Department of Pathology, Chengalpattu Medical College, Chengalpattu during the tenure of her course in M.D. Pathology from June -2014 to April-2017 under the supervision of **Dr**. **I.Vijay Sathish Kumar, M.D.,** Associate Professor , Department of Pathology, Chengalpattu Medical College and submitted in partial fulfillment of the requirements for the award of M.D. Degree in Pathology by The Tamilnadu Dr. MGR Medical University, Chennai-32. This work has not previously formed the basis for the award of a degree or diploma.

Dr. N. GUNASEKARAN M.D., D.T.C.D.
Dean,
Chengalpattu Medical College,
Chengalpattu.

Dr. **S. RAVI, M.D.,**
Professor and Head,
Department of Pathology,
Chengalpattu Medical College,
Chengalpattu.

Place: Chengalpattu

Date: 2 -09.2016.

DECLARATION

I hereby declare that the dissertation entitled "**STUDY OF STROMAL EXPRESSION OF CD 10 IN BREAST CARCINOMA AND ITS ROLE AS A PROGNOSTIC MARKER**" was done by me in the Department of Pathology, Chengalpattu Medical College during the tenure of my course in M.D. Pathology from June -2014 to April-2017 under the guidance and supervision of **Dr.I.Vijay Sathish Kumar, M.D**., Associate Professor, Department of Pathology, Chengalpattu Medical College.

This dissertation is submitted to The Tamilnadu Dr.MGR Medical University, Chennai-32 towards the partial fulfillment of the requirement for the award of M.D. Degree in Pathology.

I have not submitted this dissertation on any previous occasion to any University for the award of any degree.

Place: Chengalpattu

Date: 2 .09.2016 **Dr. Subashini. S**

CERTIFICATE FROM THE GUIDE

This is to certify that the dissertation entitled "**STUDY OF STROMAL EXPRESSION OF CD 10 IN BREAST CARCINOMA AND ITS ROLE AS A PROGNOSTIC MARKER**" submitted by the candidate Dr.Subshini S in partial fulfillment for the award of the degree of Doctor of Medicine in Pathology by The Tamilnadu Dr. M.G.R. Medical University, Chennai-32 is a record of original and bonafide work done by her under my guidance and supervision in the Department of Pathology, Chengalpattu Medical College, Chengalpattu during the tenure of her course in M.D. Pathology from June -2014 to April-2017, submitted in partial fulfillment of the requirements for the award of M.D. Degree in Pathology by The Tamilnadu Dr. MGR Medical University, Chennai-32.

Place:Chengalpattu
Date:2 .09.2016

Dr I.Vijay Sathish Kumar, M.D.,
Associate Professor,
Department of Pathology,
Chengalpattu Medical College,
Chengalpattu.

ACKNOWLEDGEMENT

First and foremost I thank the almighty for his blessings bestowed on me.

I would like to express high regards and gratitude to our respectable Dean **Dr. N. Gunasekaran. M.D. D.T.C.D.,** Chengalpattu Medical College & Hospital, Chengalpattu for granting permission to conduct this study.

I would like to take this opportunity to express my heartfelt gratitude to **Dr. S. Ravi, M.D.**, Professor and Head of the Department of Pathology, Chengalpattu Medical College, Chengalpattu for his keen interest, constant encouragement, guidance and valuable suggestions throughout this study.

My sincere gratitude for my guide **Dr. I. Vijay Sathish Kumar, M.D.,** Associate Professor of Pathology, Chengalpattu Medical College for his immense help, valuable suggestions, support and guidance to perform this study. His constant motivation and drive were the key factors for the construction of this study.

I am extremely thankful to **Dr. S Sasikala, M.D.,** Associate Professor of Pathology, Chengalpattu Medical College, Chengalpattu, who has extended her encouragement, guidance and valuable suggestions throughout the study.

I would like to express my sincere gratitude **Dr. S Premalaltha, M.D.,** Associate Professor of Pathology, Chengalpattu Medical College, Chengalpattu for her support, valuable guidance, immense help and timely advices towards the completion of my study. I am extremely grateful to her.

I would also like to express my gratitude to **Dr. G. Selambigai**, Associate Professor of Pathology, Government Omanthoorar Medical College,Chennai and **Dr. K.R. Mohan** Associate Professor of Pathology, Chengalpattu for the guidance and valuable suggestions.

It gives me an immense pleasure to thank **Dr.M.Kuzhalmozhi,M.D., Dr.S.Suryalakshmi,M.D., Dr.G.DeviPriya,M.D., Dr.V.Palaniappan,M.D., Dr.M.Malathy,MD., Dr.V.Dhamotharan,MD., Dr. D. Pushpalatha M.D., Dr. C. Arun Prabhakaran, M.D., Dr P.S.Vamitha, M.D.** Assistant Professors, **and Dr. M Sivashankari, D.C.P. Dr P.R. Shanmugapriya DCP.,** Tutors, Department of Pathology, Chengalpattu Medical College, who have extended their valuable guidance and support during the study.

I am grateful to all the faculty members and my colleagues, Department of Pathology of Chengalpattu Medical College, Chengalpattu for their constant support during the study.

Above all I am thankful to my technical staff members of Department of Pathology for their kind and selfless help for my study, without which it would have been difficult to complete my study.

To my lovable parents and my sister, I express my gratitude for their extreme patience and tireless support and being my backbone while pursuing this study.

Last, but not the least I am indebted to all the patients who made it possible for me to carry out this study. I extend all my support and wishes for their healthy lives.

CONTENTS

LIST OF TABLES

LIST OF GRAPHS

LIST OF FIGURES

INSTITUTIONAL ETHICS COMMITTEE

CHENGALPATTU MEDICAL COLLEGE , CHENGALPATTU

APPROVAL OF ETHICAL COMMITTEE

To

Dr. Subashini S,
1st Year PG student (Pathology),
Chengalpattu Medical College,
Chengalpattu

Dear Dr.

The Institutional Ethical Committee of Chengalpattu Medical College reviewed and discussed your application to conduct the clinical / dissertation work entitled

STUDY OF STROMAL EXPRESSION OF CD 10 IN BREAST CARCINOMA AND ITS ROLE AS A PROGNOSTIC MARKER

ON 19.02.2015

The following documents reviewed

1. Trial protocol, dated _____version no
2. Patient information sheet and informed consent form in English and / or vernacular language.
3. Investigators Brochure, dated _____version
4. Principal Investigators current CV
5. Investigators undertaking

The following members of the Ethics committee were present at the meeting held on

Date 19.02.2015 Time 11.00 am Place Chengalpattu Medical College

Approved_____Chairman Ethics Committee

_____Member secretary of Ethics Committee.

Name of each member with designation:-

Clinical Members

1. Dr.K.Srinivasagalu MD.,
   Prof & HOD of Medicine, CHMC

2. Dr.C.Srinivasan MS.,
   Prof & HOD of Surgery, CHMC

Biological Scientist

3. Dr.K.Baskaran MD.,
   Asso Prof of Pharmacology, CHMC

Non Clinical Member

4. Dr.P.Parasakthi MD
   Prof & HOD of Forensic Medicine,CHMC

5. Member from Nongovernmental
   Voluntary Organisation : Mr.P.Durairaj

6. Philosopher : Mr.K.S.Ramprasad

7. Lawyer : Lr. I. M. Karimala Basha

8. Layperson : Mr.Dilli

We approve the clinical trial to be conducted in its presented form

The Institutional Ethics Committee expects to be informed about the progress of the study and any SAE occurring in the course of the study, any changes in protocol and patient information / informed consent and asks to provide copy of final report.

Yours sincerely

19/2/15

Member secretary, Ethics Committee

Congratulations - your submission is complete! This is your digital receipt. You can print a copy of this receipt from within the Document Viewer.

Author:
201413502 M.d. (pathology) Subashini S

Assignment title:
2015-2015 plagiarism

Submission title:
STUDY OF STROMAL EXPRESSION OF CD 10 IN BREAST CARCINOMA AND ITS ROLE AS A PROGNOSTIC MARKER

File name:
thesis.docx

File size:
5.51M

Page count:
100

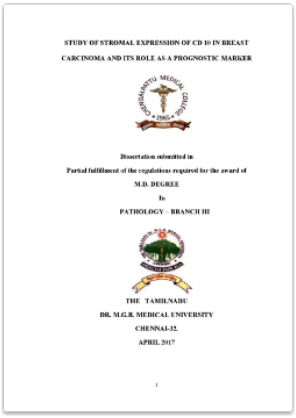
Word count:
15973

Character count:
87923

Submission date:
20-Sep-2016 01:29 IST

Submission ID:
706659241

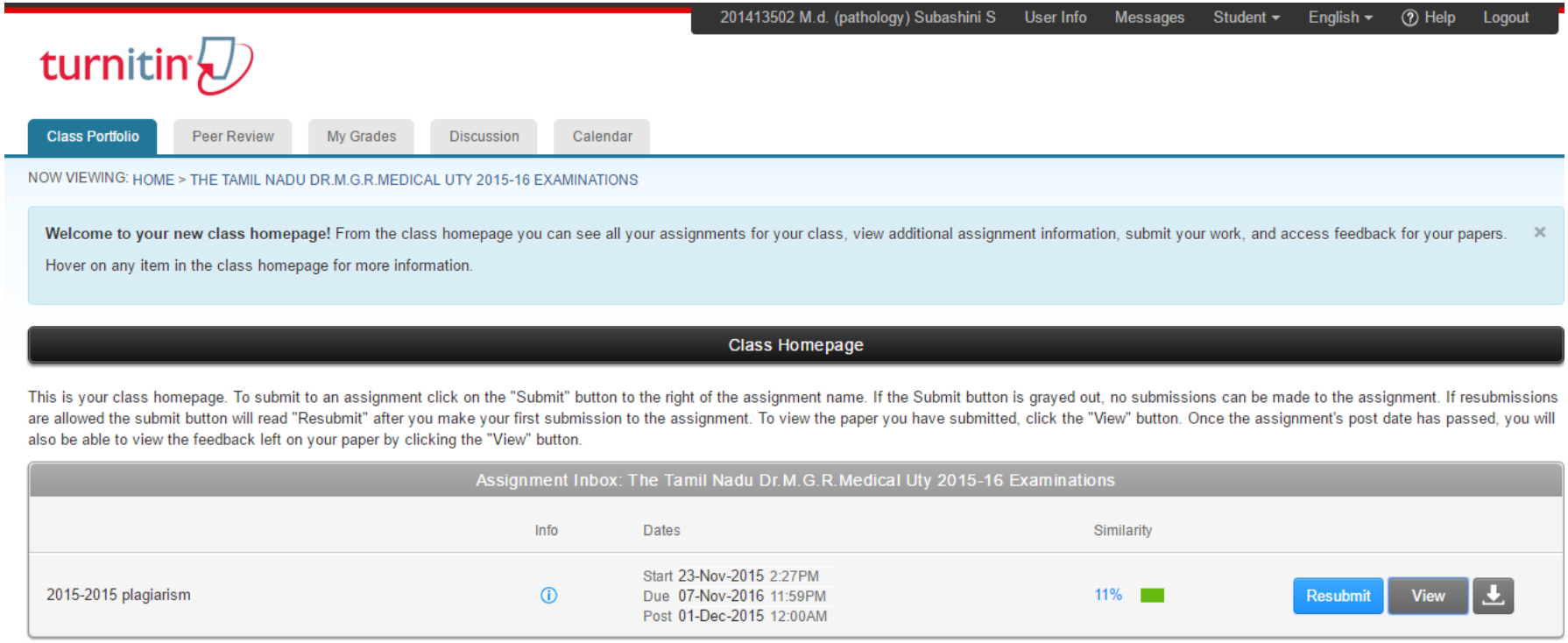
« Page 1 »



STUDY OF STROMAL EXPRESSION OF CD 10 IN BREAST CARCINOMA AND ITS ROLE AS A PROGNOSTIC MARKER

Dissertation submitted in
Partial fulfillment of the regulations required for the award of
M.D. DEGREE
In
PATHOLOGY - BRANCH III

THE TAMILNADU
DR. M.G.R. MEDICAL UNIVERSITY
CHENNAI-32.
APRIL 2017


201413502 M.d. (pathology) Subashini S
User Info
Messages
Student
English
Help
Logout
turnitin
Class Portfolio
Peer Review
My Grades
Discussion
Calendar
NOW VIEWING: HOME > THE TAMIL NADU DR.M.G.R.MEDICAL UTY 2015-16 EXAMINATIONS
Welcome to your new class homepage! From the class homepage you can see all your assignments for your class, view additional assignment information, submit your work, and access feedback for your papers.
Hover on any item in the class homepage for more information.
Class Homepage
This is your class homepage. To submit to an assignment click on the "Submit" button to the right of the assignment name. If the Submit button is grayed out, no submissions can be made to the assignment. If resubmissions are allowed the submit button will read "Resubmit" after you make your first submission to the assignment. To view the paper you have submitted, click the "View" button. Once the assignment's post date has passed, you will also be able to view the feedback left on your paper by clicking the "View" button.
Assignment Inbox: The Tamil Nadu Dr.M.G.R.Medical Uty 2015-16 Examinations
Info
Dates
Similarity
2015-2015 plagiarism
Start 23-Nov-2015 2:27PM
Due 07-Nov-2016 11:59PM
Post 01-Dec-2015 12:00AM
11%
Resubmit
View

INTRODUCTION

Breast carcinoma is the most common cancer among women with no regional variations and the second most common cancer worldwide. It accounts for an estimated 167,000cases /year (2012) worldwide. It is the fifth leading cause of cancer death worldwide accounting for 522,000 deaths/year (2012). It also tops the list of cancer death among women, living in under developed countries [1,2]. Initially a lot of stigma prevailed on seeking medical advice for breast disease. But later, due to efforts of many governmental and nongovernmental organisations, a lot of awareness has spread among women thereby increasing the detection rate of breast carcinoma, although to a lesser extent than expected.

With the introduction of Triple test approach (which includes clinical examination, mammography and fine needle aspiration cytology) a more accurate evaluation (sensitivity of 95% and positive predictive value of 100%) [3] of palpable breast lesions was made possible, helping to bring down the anxiety of patients and allowing a more planned management from the consultant's side. These awareness programmes have indeed set a trend towards a more younger age at diagnosis [4], but more alarming is the incidence of aggressive tumors in this age group (ER -ve, PR -ve, HER2/ neu +ve or triple negative tumors) [5,6]. The need of the hour is to focus on these aggressive tumors as they are increasing in incidence.

Nevertheless a lot of improvement has occurred in the arena of management of breast carcinoma from breast conservation surgeries, neo adjuvant chemotherapy, typing of tumor based on estrogen Receptor, progesterone Receptor and HER2/neu over expressions to targeted therapies against them and incorporation of all these into standard protocols of treatment.

The mortality behind breast carcinoma is attributed to metastatic disease, and a better understanding of the molecular basis of metastatic disease would have practical implications in the clinical areas of diagnosis, treatment and prognosis.

Newer researches are thus gaining momentum to crack down and throw light on pathways at molecular level which enhance invasion and metastasis, thereby implicating aggressiveness and development of products or drugs which can target these molecules, thereby reducing the mortality rate and helping the patient have disease free life, bringing the morbidity and increasing the quality of life.

The process of tumor spread is dynamic, involving many intracellular molecular changes and genetic alterations including overexpression or mutation which normally regulate cell proliferation and differentiation, such as hormone receptors, growth factors, oncoproteins and tumor suppressor genes.

The female breast comprises of 2 types of epithelial cells: myoepithelial cells and luminal (secretory) cells arranged in lobules containing these acini. The intralobular stroma contains fibroblast like cells, is more vascular and hormone dependent. The interlobular stroma is hormone independent. The focus of recent research is on the nature and molecular signature of stroma such as fibrosis, role of metalloproteinase, and appearance of elastic fibres in correlation with the invasion, metastasis and whether they indirectly dictate the prognosis, though it's still not clearly evident.

CD10 is a 90-110 kilo Dalton cell surface metalloproteinase, also called Common Acute Lymphoblastic Leukemic Antigen (CALLA). It belongs to the group of matrix metalloproteinase and cleaves bioactive peptides. It is expressed by myoepithelial cells of normal breast and also expressed by lymphocytes, endometrial stroma cells, etc. CD10 is gaining importance recently in tumors like renal cell carcinoma, endometrial stromal sarcoma, cannalicular pattern of hepatocellular carcinoma apart from Acute Lymphoblastic Leukaemia from where it got its name CALLA.

Studies done in the last decade have found out and established the disappearance of CD 10 expressed by myoepithelial cells in malignant tumors[7]. Recent evidence in literature points to few studies which have discovered stromal expression of CD 10 and correlated this expression of CD 10 in stromal cells of breast carcinoma with other prognostic factors [8, 9].

The pathogenesis proposed for expression is the interaction of tumor cells with stromal cells and extra cellular matrix leading to CD10 expression in stroma, which then acts to degrade extra cellular matrix and collagen, providing a microenvironment favourable for invasion and metastasis.

Attempts to establish a prognosis based upon generally accepted factors such as tumor size, grade and axillary lymph node status are successful to some extent, but still fail to accurately predict the clinical course for all patients because the intrinsic metastatic potential of cancer cells vary in each individual patient. Many studies have shown that varied survival rates among similar group of patients, explaining the heterogeneity of breast cancers and the need for further molecular research and a better stratification of patients. Hence the search for new prognostic marker that could elucidate more effectively the metastasizing potential and thereby get integrated as additional prognostic factor in the treatment algorithm of breast carcinomas remains an important goal of this study.

The aim of this study is to analyse the stromal expression of CD 10 in breast carcinoma patients and its relationship with invasion, metastasis and survival rates indirectly, by using Nottingham's prognostic index, Mitotic rate and tumor grade. Thus the role of stromal expression of CD 10 is evaluated as a prognostic marker in breast carcinoma.

AIMS AND OBJECTIVES

1. To study the stromal expression of CD 10 antigen in patients diagnosed as invasive ductal breast carcinoma by histopathological examination.

2. To calculate the Nottingham s Prognostic index, Mitotic rate and histopathological grade of invasive ductal breast carcinoma for the same group of patients.

3. To analyse and correlate the CD10 stromal expression in invasive ductal carcinoma of breast, with the calculated Nottingham's Prognostic index, assessed Mitotic rate and tumor grade of the patients.

REVIEW OF LITERATURE

Breast or mammary glands are the organs which help in nursing the new born**.** It is priceless for the new born with regards to adequate nutrition, transfer of immunity, and bonding, and also advantageous for the mother with concern to postpartum uterine involution.

Throughout the reproductive age, under the influence of multifarious hormones, there are continuous changes in the histologically structure of breast. Thus a complete understanding of the histology, physiological changes of the breast during development and in reproductive age is vital to study and acquire a full picture of the pathophysiology of the breast and is thus inevitable in the diagnosis and management of neoplastic disorders.

Embryological development

Breast develops from interactions between ectoderm and mesenchyme. During the 5^{th} week of intrauterine life there is appearance of epidermal thickenings on the ventral surface, along the ectodermal primitive milk line/galactic band/milk streak extending from axilla to groin. Most of the ridges disappear by ninth week of intrauterine life except at the pectoral region.

At seven to eight weeks of intrauterine life the thickening at pectoral region invaginates (milk hill stage), followed by tridimensional growth (globular stage). From 12 to 16 weeks of intrauterine life there is

differentiation of mesenchymal cells into the smooth muscle of the nipple and areola. Epithelial cords form buds and branch to form 15 to 25 cords (branching stage) at 16 weeks of intrauterine life; they represent the secretory alveoli. Differentiation of the hair follicle, sebaceous gland, and sweat gland elements ensues, but only the sweat glands develop completely and sebaceous glands are not accompanied by hair follicles (secondary development).

After twenty seven weeks of intrauterine life, the hormones enter fetal circulation inducing canalization of the epithelial tissues (canalization stage). This continues, to form 15 to 25 mammary ducts. After thirty two weeks of gestation there is the development of lobulo alveolar structures [10-17]. During this time nipple forms from stratum spinosum initially as a pit, everting at birth [18].

Papillary dermis surrounding the cords form fibrous tissue surrounding the ducts and its branches. The reticular dermis forms the suspensory ligaments of Astley Cooper attaching breast to the dermis. The myoepithelial cells develop around 23-28 weeks of gestation and important in branching of glandular tissue [19-24].

Breast Anatomy

The breasts or mammary glands are modified sweat glands, which lies in the superficial fascia, anterior to the pectoral muscles and the thoracic wall. The breast lies on deep fascia related to the Pectoralis major muscle and other

surrounding muscles including serratus anterior and external oblique. The retromammary space is a layer of loose connective tissue that separates the breast from the deep fascia and provides some degree of movement over underlying structures [19, 20].

The attached surface of each breast extends vertically from second rib to sixth rib, and transversely from the lateral border of sternum to the midaxillary line. The superolateral region shows continuation of breast tissue towards the axilla along the inferolateral edge of pectoralis major muscle which is called the axillary tail of Spence. The mammary glands have a conical shape with base being 10-12 cm in diameter with thickness 5-7cm. The average weight of non- lactating breast varies between 150-225 g and lactating breast more than 500g [25-27].

The breast contains lobules of glandular tissue which converge to form 15 to 20 lactiferous ducts, which are arranged radially around and open independently onto the nipple. The nipple is surrounded by a circular pigmented area of skin termed the areola. The skin covering the nipple and areola have convoluted surface containing numerous sweat and sebaceous glands which open directly to the skin surface. Histologically they have long dermal papillae. Numerous sensory nerve endings is the rule in nipple with areola having few. There is no subcutaneous fat below the nipple areola complex and it contains only radial and circular smooth muscles which help in erection of nipple when excited [28].

Arterial supply

The mammary gland is extremely vascular. It is supplied by branches of the following arteries:

- Predominantly by perforating branches of Internal thoracic (mammary) artery (a branch of sub-clavian artery).
- The lateral thoracic, superior thoracic and acromiothoracic branches of the axillary artery.
- Lateral branches of the posterior intercostal artery.

Venous drainage

Veins draining the breast run parallel to the arteries. The breast has a well - developed superficial venous system forming extensive anastomotic network called circulus venosus and a deep venous system, all of which ultimately drain into the axillary vein, internal thoracic vein and perforating branches of posterior intercostal veins. The drainage to posterior intercostal vein serves as a route of spread to vertebral venous plexus [29, 30].

Innervation

Innervation of the breast is through the anterior and lateral cutaneous branches of the second to sixth intercostal nerves and the supraclavicular nerves. The nipple is innervated by the fourth intercostal nerve [28].

Lymphatic Drainage

The lymphatic drainage of the breast is of paramount importance because of its key role in metastasis and thereby altering the overall prognosis of breast carcinoma patients. Lymph passes from the nipple and areola to the subareolar lymphatic plexus, and from there to the deep plexus. This is called centrifugal flow. Subsequently most of the lymph from deep plexus, approximately 97% drains into the axillary nodes whereas remaining 3 % to the internal mammary nodes.

Lymphatic vessels in the skin of the breast, except the nipple and areola, drain into the axillary nodes, inferior deep cervical nodes and inferior clavicular nodes and to the parasternal nodes bilaterally. Superficial lymphatics have communication with the opposite breast and anterior abdominal nodes, incriminating possibilities of metastasis to these sites.

Glandular tissue drains to axillary, sub clavicular and internal mammary nodes. No specific quadrant drainage to each has been proved till now, though upper outer quadrant is claimed to predominantly drain to axillary nodes and inner quadrants to internal mammary nodes. Also some lymph may drain directly to the interpectoral, deltopectoral, supraclavicular or inferior deep cervical nodes.

Lymph from the axillary nodes further drain into infraclavicular and supraclavicular nodes. Lymph from these nodes finally drain into

the subclavian lymphatic trunk, which also receives lymphatics from upper limb. Lymphatics from parasternal nodes drain into the bronchomediastinal trunk**,** which also receives lymphatics from the thoracic viscera. The trunks drain into the brachiocephalic vein, which is formed by joining of the internal jugular vein and the subclavian vein [30-40].

Axillary nodes have been divided into six groups namely: Anterior pectoral nodes, Scapular nodes, Central nodes, Interpectoral nodes, Axillary vein nodes and Sub clavicular nodes.

Three levels of axillary nodes of paramount importance in staging include:

Level I nodes: lateral to lateral border of pectoralis minor muscle, corresponds to low axilla group.

Level II nodes: under the pectoralis minor muscle, corresponds to mid axilla group.

Level III nodes: medial to medial border of pectoralis muscle, corresponds to apical axillary group [18].

Histology

The basic functional unit of breast is a complex branching structure comprising of two components: the terminal duct lobular unit (TDLU) and the large duct system. The TDLU denotes the lobule and terminal ductule and it is the secretory portion of the gland. It connects with the duct system which

starts as subsegmental duct, leading onto a segmental duct and further to a collecting duct, which finally opens onto the nipple. Lactiferous sinus is dilatation in the lactiferous ducts just before entering into the nipple.[41, 42]

The entire ductal-lobular system of the breast is a compound tubulo alveolar gland, lined by specialised two cell type epithelium: the inner epithelium which has secretory and absorptive functions and the outer myoepithelial cells function as contractile elements helping in flow of milk from sites of secretion to the ducts eventually to the nipple. The luminal epithelial cells are cuboidal to columnar with pale eosinophilic cytoplasm and relatively uniform oval nuclei. The outer myoepithelial cell layer is cytologically represented by either flattened cells with compressed nuclei or prominent epithelioid cells with abundant clear cytoplasm. [42-45]

The intralobular stroma can be influenced hormonally, is more cellular whereas the interlobular stroma is more collagenised, less cellular and hormonally independent. [43]

PHYSIOLOGY

Mammary glandular development and functions are instigated predominantly by hormonal stimuli, including oestrogen, progesterone, prolactin, oxytocin, thyroid hormones, cortisol and growth hormone. Oestrogen and prolactin signal trophic changes to the breast and play an essential part in normal development and functioning of mammary glands.

The external morphologic appearance of developing breast has been divided into 5 phases by Tanner. The term alveolus refers to resting mammary glands whereas acini refers to fully developed secretory unit of pregnancy and lactation.

Oestrogen initiates the ductal development in the breast whereas progesterone primarily stimulates the differentiation of epithelial cells and lobular development. Progesterone also reduces the oestrogen binding in mammary epithelium and limits the proliferation of tubular unit. Prolactin is the chief hormonal stimulus for lactogenesis in late pregnancy and in the postpartum period. Prolactin increases the number of oestrogen receptors and encourages epithelial cells to act in harmony with ductular and lobuloalveolar growth. Ductular growth is further stimulated by Growth hormone and glucocorticoids. Insulin and growth hormone are involved in the lobuloalveolar differentiation and growth. The cells lying at the end of the terminal ducts give rise to the lobules.[43,46,47]

Hypothalamus secretes gonadotropin releasing hormone (GnRH) which stimulates the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the basophilic cells of anterior pituitary. LH and FSH further stimulates the ovary to secrete oestrogen and progesterone. Oestrogen and progesterone apart from the usual physiologic functions also serve as positive and negative feedback control for the release of LH, FSH and GnRH. These hormones act throughout the reproductive age on the

mammary glands and help in the maintenance of lobulo alveolar units and appropriate functioning of the mammary gland.

With birth, there is a fall in the levels of circulating oestrogen and progesterone. It continues to remain low under the control of hypothalamic-pituitary axis during the childhood. With onset of puberty there is an increase in the central drive of hypothalamus with a decrease in sensitivity to the negative feedback control by oestrogen and progesterone. The physiologic events thus move on to produce an increase in GnRH secretion which in turn increases FSH and LH secretion with a final outcome of increase in ovarian oestrogen and progesterone secretion. Following development of positive feedback by oestrogen, the menstrual cycle gets established.[17,43]

Cyclic Changes during the Menstrual Cycle

There are great range of variations in breast volume during the menstrual cycle. Volume is greatest in the second half of the cycle. Premenstrual time period is characterised by increase in size, nodularity, density and sensitivity. Progesterone functions to stimulate glandular growth in the luteal phase. Changes in the mitotic rate of glandular components are more in the luteal phase than in the follicular phase. The premenstrual increase in volume is attributed to increase in size of the lobule without epithelial proliferation. There is luminal dilatation associated with the increase in size of ducts and alveoli. Interlobular edema is also seen during

this phase of menstrual cycle. Parenchymal engorgement and edema subside with onset of menses .[17,43,48,49]

Changes during pregnancy

Pregnancy is associated with decrease in amount of connective tissue and infiltration of stroma by cells including plasma cells, lymphocytes and eosinophils. Degree of development varies with each lobule. The second trimester sees the lobular development surpassing ductular one. During parturition there is prominent alveolar development accompanied by hypertrophy of secretory cells and accumulation of secretory products in the alveoli. Donne corpuscles are collection of lymphocytes and desquamated alveolar cells found in the colostrum. [43,49-51]

Changes in menopause

Following menopause the ovarian function decreases leading onto regression of epithelial structures and stroma. There is decrease in both the ducts and the lobules. Increase in fatty tissue along with shrinkage of lobular component is also evident.[17,43,52]

History

The oldest known description of breast cancer was discovered in Egypt and dates back to approximately 1600 BC. This was Edwin Smith Surgical Papyrus believed to contain the first reference of breast cancer. This was

presented in the New York Historical Society and case 45 gives earliest record as an ailment for which there is no treatment. It was suggested to use cautery and knife excision as treatment.[17]

Epidemiology

Breast carcinoma is the most common cancer diagnosed among women, with the worldwide statistics of 167,000 cases / year for the 2012. The incidence of breast cancer is approximately 25.2% with a mortality rate of 14.7% for the entire world. In India, the burden amounts to 145,000 cases/ year (2012) and we lose around 70,000 patients / year (2012) due to breast carcinoma. Analysis of the recent statistics in comparison with earlier, point towards a higher incidence rate by 20% and a higher mortality rate of 14%. The key attributes for higher mortality rate have been suggested as delayed detection rate and less accessible treatment facilities.[1,2]

Risk factors

Breast carcinoma is a multifactorial disease. The presence of a genetic mutation per se cannot point to development of a carcinoma but requires a complex interaction between environmental, genetic and hormonal factors. Risk factors can be grouped as:

Factors important in populations:

Parity

Age at menarche and menopause

Breast feeding

Age at first birth

Alcohol consumption

Exogenous hormone use or exposure

Factors important in individual patients:

Age

History of previous breast carcinoma

Family history

Histologic risk factors:

Atypical lobular hyperplasia

Atypical ductal hyperplasia

Proliferative breast disease

Lobular carcinoma insitu

Regional variations: Though the exact etiopathogenesis is not known, there exists variations in the incidence of carcinoma in different localities. Higher rates are observed in northern part of America and northern Europe, Asia and Africa have recorded lower rates.

But the recent statistics show a slightly higher incidence in under developed countries compared to more developed countries.[54,55]

Menstrual and reproductive history: Most of these histories reveal an association with early menarche, nulliparity, late age at first child birth and

late menopause with one year delay of menarche, the breast cancer frequency decreasing by 10-20%. Both age of onset of menarche and regular cycles influence the risk of breast cancer. Regular cycles following menarche show 4 fold greater increased risk compared to late menarche and long duration of irregular cycles. The effect of early menarche on breast cancer risk may be explained by high estrogen exposure.

Women with late age at menopause 55 years have a relative risk of 1.48 compared to menopausal age of 45 years. The effects are presumed to be due to high estrogen exposure and circulating levels.[53,54,56-59]

Parity: Younger age at first pregnancy decreases risk of breast cancer. 17-41% reduction in breast cancer risk is seen in parous women when compared to nulliparous and single women. It is explained by proliferative changes in pregnancy. The reduction is due to maximum differentiation in breast parenchyma which decreases further chances of DNA damage. Both first pregnancy and immediate period show a slightly higher incidence of breast carcinoma, attributed to hormonal levels.

Women more than 35 years of age have 60% increased risk in breast cancer than those who are less than 18 years of age at first pregnancy.[53]

Breast feeding: Breast feeding further reduces the risk of breast cancer in parous women. There is about 12% decrease in relative risk of breast cancer for women who breast fed for 12 months. This reduction percentage is

increased upto 7% for each birth in high parity females. Breastfeeding is thought to decrease breast cancer risk by lessening the total number of menstrual cycles and consequently cumulative ovarian hormone exposure.[53,54]

Diet: Researches show inconclusive results of any diet factors associated with breast carcinoma. Nevertheless high intake of antioxidant lessens the risk, which includes β carotene and lycopene. American Cancer Society recommendations to decrease risk include more than 5 servings of fruits or vegetable; including whole grains than refined products; decreasing consumption of red meat .[53]

Alcohol consumption: Most important modifiable risk factor: more than one drink per day was consistently associated with development of breast carcinoma. The relative risk was 9% higher for every 10 g of alcohol intake. The underlying mechanisms include increased serum and urinary estrogen and decreased serum hormone binding globin and also acetaldehyde production from ethanol inhibits DNA repair.[53]

Estrogens and Androgens

Estrogen induces cell proliferation in the breast. In premenopausal women, source of estrogen is ovarian whereas for post-menopausal, aromatization from androstenedione in adipose tissue. Proliferation of breast epithelium is twofold higher in luteal phase.

Estradiol and estrone sulfate are the types of estrogen implicated in breast cancer development. The 17β estradiol is the most functionally active form of estrogen. Estradiol circulates in the blood either as free hormone or bound to sex hormone binding globulin (SHBG) and albumin. Major circulating estrogen is estrone sulfate. They are the major resource of estrogen from adipose tissue in postmenopausal females.

Androgens such as testosterone and androstenedione can be aromatized into estrogens, either in the ovaries or in adipose tissues. Testosterone is positively associated with post-menopausal breast carcinoma.

Breast cancer risk is directly proportional to the levels of serum concentrations of sex hormones .Serum estradiol levels are shown to be less in Asian women regardless of menopausal status. These differences may be due to reduced number of ovulatory cycles as a result of late age at menarche, higher parity, frequent breast feeding, breast feeding for longer durations, and early age at menopause. In postmenopausal women, weight is directly proportional to plasma levels of estrone and estradiol.[53,54,59,60]

Hormone replacement therapy: Invasive breast carcinoma is higher in women using Hormone replacement therapy. But this risk depends on few factors. Risk for women who stopped taking hormone replacement therapy five years back equals women not taken hormone replacement therapy. Higher risk is noticed if hormone replacement is taken for five years or more.

The major consequence of hormone replacement therapy is promotion of cancer growth rather than direct effect.

The risk of developing breast cancer is increased by 2.3% for each year among women using hormone replacement therapy currently, or who have stopped within one to four years. Whereas the relative risk is 1.35 for women who had used hormone replacement therapy for more than 5 years. Also 6% increased risk is associated with only estrogen use which increases to 24 % with combined estrogen and progesterone use.[53,54,61-64]

Body weight and obesity

The risk of obesity depends on menopausal status. Obesity in post-menopausal women increases the risk of developing breast carcinoma whereas in premenopausal it has inverse relationship. It has been attributed that in premenopausal women, obesity may cause anovulation and decrease the progesterone level. Leptin increases with increasing fat stores, inhibits ovarian estrogen production, and can thereby decrease breast cancer development. Relative risk is 0.54 in premenopausal women with BMI more than 31kg/m2 compared to BMI 21kg/m2. Higher levels of physical activity convenes a 10- 60% lesser risk.

Obesity increases breast cancer risk in postmenopausal women by increasing levels of endogenous estrogen. Also, sex-hormone-binding globulin levels fall when BMI is increased, thus increasing the levels of

free estradiol. Also action on insulin and insulin like growth factors have been observed which are mitogens to breast epithelium along with estrogen .[53,54,65-67]

Age: There is a steep rising curve with increasing age.

Family history: Familial breast carcinoma is a well-known entity, the responsible genes and cytoband include BRCA 1, BRCA 2, CHEK 2, FANC GENES, CDH 1 ATM, TP53, PTEN, STK11, MSH1, MSH3, MSH6, MLH1, PMS1 AND PMS2 . BRCA 1 localises to chromosome 17 and BRCA 2 localises to chromosome 13. Contradictory to earlier thoughts, breast carcinoma due to BRCA genetic mutations account to only 16% of all familial breast carcinomas. The lifetime risk is increased by 2 to 3 times if a first degree relative is affected. But also depends on the age of diagnosis of the relative, if diagnosis was at < 40 years relative risk is 6; if more than 1 relative then relative risk is 3-4 .[53,54,68-70]

Some other syndrome associated with breast carcinoma include Li-Fraumeni syndrome, where there is increased risk of development and also early onset of many cancers including breast cancer. They have mutations affecting p53 tumor suppressor gene. In Ataxia telangiectasia, there is 100 fold increase in breast cancer risk, being an autosomal recessive syndrome due to mutations affecting DNA repair genes (71). Women with Cowden

disease having mutation in the PTEN tumor suppressor gene, develop breast cancer before 50 years of age .

Benign breast disease and contralateral breast carcinoma: 3- 4 fold increase in breast carcinoma occurs if the other side breast was involved. Whereas the risk for benign breast disease ranges from 1.5 to 3. Certain types of benign breast diseases have increased risk of breast cancer. There is 1.5 fold increased risk of breast cancer for those women with benign breast disease without hyperplasia compared to normal population. The risk of breast cancer among women with hyperplasia varies with whether atypia is present or not. Atypical hyperplasia increases the risk by 2.6 fold as compared to 1.8 fold increased risk in hyperplasia without atypia.

The breast cancer risk associated with benign breast disease differs by menopausal status. Atypia in premenopausal women have higher relative risk of breast cancer than in post-menopausal women .[53,54,71-75]

Ionizing radiation: Has increased risk of developing breast cancer. Studies are mostly based on incidences in atomic bomb explosion localities. Younger women less than 40 years of age had greater risk than older women. The effect of very low doses such as those incurred in occupational exposures is not harmful; higher risk if exposure has occurred at time period of breast development namely, with age of less than 5 years the risk was 9 fold higher.

This is due to increasing the susceptibility of breast tissue to tumor promoting effects of steroid hormones.[53,54,76]

Socioeconomic Status

High socioeconomic status is associated with increased risk of breast carcinoma both at individual and community level. This is also reflected by higher incidence in developed countries. The higher breast cancer risk is attributed to greater exposure to breast cancer risk factors including late age at first pregnancy, having few or no children, and more frequent use of oral contraceptives and hormone therapy. The baseline of all these risk factors is a strong / or a prolonged oestrogen exposure beside genetic alterations.

The key stone in search for risk factors is that by identifying modifiable risk factors, the incidence of breast carcinoma can be lowered.[53,54]

The WHO classification of breast carcinoma [77] has been given at ANNEXURE I.

Modalities available for diagnosis

Breast carcinoma most commonly presents as mass in the breast. Other symptoms include pain and bloody nipple discharge. History of rapid growth can be easily ascertained from these patients. Very rarely is a tumor occult or with presentation of skin involvement such as edema and redness of the overlying skin is seen.

Physical Examination

Simple, useful and informative test. Though there are limitations with diagnostic accuracy having a wide range of differential diagnosis including fibroadenoma, phylloides, etc., still holds good as a highly significant simple testing modality.

Radiological tests

Mammography: A valuable screening tool is advised for older women as younger women have more breast tissue which hinders with the results of this imaging modality. Hence younger women are advised nuclear magnetic resonance imaging. Current recommendations suggest screening with mammography after 40-50 years of age. Rarely useful for less than 35 years of age.

Most common mammography finding of invasive breast carcinoma is stellate and circular tumor mass without calcification (64%), only calcification (19%) and stellate and circular tumor mass with calcification (17 %).[77]

Mammography is also a diagnostic modality for patient with abnormal screening mammogram as follow up, palpable breast mass, nipple discharge and patient who underwent breast conservation surgeries.[78,79] Not to be forgotten during mammography - histopathology correlation is that

calcifications produced by calcium oxalate identified in mammography will not be evident in histologic sections.[54,80]

Digital mammography / Full-Field Digital Mammography (FFDM)

FFDM is a new technology that was recently approved by the FDA for breast cancer screening and diagnosis**.** They capture the images which are processed on a computer and then viewed.

BIRADS diagnostic categories

After analysing the mammographic images, radiologists classify findings into a final assessment category. The Breast Image Reporting And Data System (BIRADS) was developed by the American College of Radiology to standardize mammographic reporting. Follow up recommendations are made based on the final assessment category.[81]

Nuclear magnetic resonance imaging

Nuclear magnetic resonance imaging is a more sensitive tool for detection of multicentric breast carcinoma.[54,82,83]

Ultrasonography

Ultrasonography is another valuable tool to differentiate cystic from solid lesions.[54,84]

Cytology

Cytological examination of breast is a cheap, quick and informative test but cannot be used as confirmatory diagnostic test for breast carcinoma as it is only a supplementary test. The cytological examination can be with either Nipple secretion smears which is of limited value or aspiration from lesion which gives highly rewarding results. The drawback of cytological examination include failure of distinction between in situ and invasive carcinoma as it depends on stromal invasion and not cytology of cells.

But gaining momentum is its use in hormone receptor studies, kinetic studies and oncoproteins expression. The possible after-effects of cytological examination includes mechanical displacement of epithelium, haemorrhage and necrosis.

Needle core biopsy

Simple test, provides better information with regards to assessment of cytoarchitectural features, the assessment of stromal invasion and clear cut distinction between in situ lesion and invasive lesions are possible. It permits easier identification of microcalcifications. The gray zone of diagnosis include infiltrating lobular carcinoma and radiation induced histiocytic proliferations.

Post needle core biopsy can show evidence of haemorrhage, reactive spindle cell nodules, epidermal inclusion cysts, displacement of tumor cells.[77,85,86]

Open biopsy and frozen section

Open biopsy gives a higher diagnostic yield and accuracy. The main application of frozen section is its use in assessment of margin involvement by tumor. Frozen section is of limited value in lesions with papillary proliferations which dictate a routine haematoxylin and eosin section. Evidence based medicine has thrown light that the prognosis is not affected by delay in time period, following diagnosis by trucut biopsy and mastectomy. This has thus raised questions on using frozen section as a diagnostic modality as the limitations of frozen section include resource reduction (for haematoxylin and eosin section and hormone studies) and architectural distortion .[87-89]

Morphology: Classic No Specific Type (NST)

A firm poorly circumscribed growth with irregular stellate or crab like appearance. Chalky streaks due to duct elastosis can be seen along with necrosis and cystic degeneration. Cut surface usually reveals a grey white tumor with yellow streaks (Figure 3).[54]

Microscopic types

Classic NST (40 – 75% of reported cases)

Microscopy shows diffuse sheets, nests, cords, clusters, trabeculae and individual cells with surrounding densely fibrotic to cellular / desmoplastic stroma. Glandular/tubular differentiation can be seen, which forms a part of the grading system. The tumor cells are larger and more pleomorphic than those of the classic form of invasive lobular carcinomas, their nuclei and nucleoli are more prominent, and mitotic figures are more numerous. Foci of squamous metaplasia, apocrine metaplasia, clear cell changes, calcification (60% of cases) and necrosis may be seen. Chronic inflammatory infiltrate composed of mononuclear cells is usually seen at the interphase between tumor and stroma (Figure 4, 5,& 6).[77,90]

Immunohistochemically, the tumor cells show reactivity for low molecular weight keratin (particularly types 8, 18, and 19) and EMA. Two other important breast-related markers are mammaglobin and Gross Cystic Disease Fluid Protein -15 (GCDFP-15), the former being more sensitive but less specific than the latter.[90]

Most common variants include

Tubular carcinoma: (2%)

On microscopy, shows haphazard arrangement of irregular and angulated glands in abundant fibroblastic stroma, absence of organoid configuration with apocrine snouts lined by single layer of cells. They are usually small sized tumors and have excellent prognosis. Nuclear pleomorphism and stratification go against this diagnosis.[77,90]

Cribriform carcinoma: (0.8 3.5%)

This type has excellent prognosis microscopically, shows cribriform appearance of cells enclosing clear lumen, with stromal invasion in an angulated fashion.[77,90]

Mucinous carcinoma: (2%)

Seen in post-menopausal women. Macroscopically shows gelatinous appearing bosselated tumors with pushing margins. Microscopy shows small clusters of tumor cells floating in sea of mucin. The feature is presence of extracellular mucin which belongs to acid or neutral mucin group. The O acetylated forms of sialomucins is exclusive to type A. Type B shows neuroendocrine differentiation. It is associated with low nodal metastasis, excellent short term prognosis (figure 7, 8).[77,90]

Medullary carcinoma: (1-7%)

Most commonly seen in age group of 45-52 years. Frequent in Japanese population. Grossly shows well circumscribed mass with pushing margins. Shows minimal or no glandular differentiation. Indistinct syncytial or sheet pattern, spindle cell metaplasia, bizarre tumor giant cells, extensive necrosis, absence of calcifications along with prominent lymphoplasmacytic infiltration are the characteristic findings. Absence of mucin is a key diagnostic feature.[77,90]

Invasive papillary carcinoma (1-2%)

Associated with better prognosis and most common in whites, among postmenopausal age group. Microscopy shows delicate, blunt papillae composed of cells with amphophilic cytoplasm. Also seen are inversion of polarity and intermediate nuclear grade. The tumors are positive for oestrogens, mammaglobin, WT1 (figure 9,10).[77,90]

Apocrine carcinoma (0.3-4%)

It is composed of two types of cells microscopically. Type A shows acidophilic granular cytoplasm, whereas type B shows abundant cytoplasm with fine empty vacuoles resembling foamy histiocytes.[77]

Metaplastic carcinoma : (< 1%)

They are a group of tumors composed of admixture of adenocarcinoma with predominant areas showing spindle cells or squamous cells with or without mesenchymal differentiation.[77]

Invasive lobular carcinoma

Accounts for 5-15% of breast carcinomas. On microscopy, shows proliferation of small cells lacking cohesion, seen to infiltrate stroma in single file/linear cord pattern. Occasional intracytoplasmic mucoid inclusion can be seen. Variants include solid, alveolar and pleomorphic lobular carcinomas.[77,90]

Molecular Subtypes of Breast Carcinoma

Molecular subtyping of breast cancer is an important discovery, which has changed the treatment protocols of breast carcinoma patients thereby improving the survival rates and decreasing the morbidity**.**

Table 1: Molecular subtypes of breast carcinoma along with gene expression pattern, histological correlation and prognosis .[54]

Immuno profile	Luminal A (50%)	Luminal B (20%)	HER2/neu (15%)	Basal like (15%)
ER,PR	ER and or PR positive	ER and or PR positive	ER negative PR negative	ER negative PR negative
HER2 and others	HER2 negative Low Ki67 (<14%)	HER2 positive or negative Ki67 (>14%)	HER2 positive	Her2 negative CK5/6 and or EGFR positive
Gene expression pattern	Luminal cytokeratins with high hormone receptor expression	Luminal cytokeratins with weak to moderate hormone receptor expression	High expression of HER2	Basal epithelial genes and cytokeratins
Histological correlation/ subtypes	Tubular carcinoma, Cribriform carcinoma Low grade IDC NST Classic lobular carcinoma	IDC NST Micropapillary carcinoma	High grade IDC NST	High grade IDC NST, Medullary carcinoma, Metaplastic carcinoma
Treatment and response	Responds to Endocrine therapy	Responds to endocrine therapy not as good as Luminal A	Responds to Trastuzumab and anthracycline based chemotherapy	Platinum based therapy and PAPP inhibitors
Prognosis	Good	Not as good as for Luminal A	Poor	Poor not uniformly

Prognostic and predictive factors: Some of the important prognostic and predictive factors include:

Age: Discrepant results are available but younger age group, less than 35 years have higher lymph node positivity and higher incidence of aggressive tumors.[92,93]

Pregnancy: Breast cancer detected during pregnancy and lactation has an aggressive course, but whether per se due to pregnancy or other confounding factors like age is not confirmed.[94]

Tumor size: One of the independent prognostic factor. Tumor size of less than 10 mm has excellent prognosis. Minimally invasive carcinoma is defined by tumor size of less than 10 mm. Tumor size has been included in TNM staging of breast carcinoma and also in various prognostic indicators. Also correlates with lymph node involvement in tumors having a size more than 15 mm. In case of discrepancies in gross and microscopic size, microscopic size is considered accurate.[90,95-98]

AJCC staging and TNM classification: Most widely used prognostic indicator. Holds high regards with predicting the survival of patient and are a must in the reporting format. Given below is a detailed version of it.

STAGING OF BREAST CANCER (77).

The American Joint Committee on Cancer (AJCC) stages for breast cancer:

pTNM pathological staging

Primary tumor (pT)

pT categories correspond to T categories

TX	:	Primary tumor cannot be assessed
T0	:	No evidence of primary tumor
Tis	:	Carcinoma in situ
Tis(DCIS)	:	Ductal carcinoma in situ
Tis (LCIS)	:	Lobular carcinoma in situ
Tis (Paget s)	:	Paget disease of nipple with no tumor
T1	:	Tumor 2 cm or less in greatest dimensions
T1mic	:	micro invasion 0.1cm or less in greatest dimensions
T1a	:	more than 0.1 cm but not more than 0.5 cm in greatest dimensions
T1b	:	more than 0.5cm but not more than 1 cm in greatest dimensions
T1c	:	more than 1cm but not more than 2 cm in greatest dimensions
T2	:	Tumor more than 2 but not more than 5 cm in greatest dimensions

T3	:	Tumor more than 5cm in greatest dimensions
T4	:	Tumor of any size with direct extension to chest wall or to the skin only as described in T4 a – T4d
T4a	:	Extension to chest wall, not including only pectoralis muscle invasion/adherence
T4b	:	Edema or Ulceration of skin of breast or satellite skin nodules
T4c	:	both of the above (T4a and T4b)
T4d	:	Inflammatory carcinoma

Regional lymph nodes (pN)

pNX	:	cannot be assessed
pN0	:	No regional lymph node metastasis (RLN)
pN0(i-)	:	No 'RLN' metastasis identified histologically, negative IHC
pN0(i+)	:	Malignant cells in 'RLN' less than 0.2 mm (H&E or IHC)
pN0 (mol-)	:	No RLN metastasis histologically, negative molecular findings (RT-PCR)
pN0(mol+)	:	Positive molecular findings (RT-PCR) but no RLN metastasis detected histologically or by IHC
pN1mi	:	Micro metastasis (greater than 0.2 mm and /or more than 200 cells but none greater than 2.0mm)
pN1a	:	Metastases in 1 - 3 ipsilateral axillary lymph nodes, and or atleast one metastases greater than 2.0 mm

pN1b : Metastases in internal mammary nodes with micro metastases or macro metastases detected by sentinel lymph node biopsy but not detected clinically

pN1c : Metastases in 1 to 3 lymph nodes and in internal mammary nodes with micro metastases or macro metastases detected by sentinel lymph node biopsy but not detected clinically

pN2 : Metastasis in 4-9 axillary lymph nodes or in clinically apparent ipsilateral lymph nodes in the absence of axillary lymph node metastasis

pN2a : Metastases in 4-9 axillary lymph nodes (atleast one tumor deposit greater than 2.0 mm).

pN2b : Metastases in clinically apparent internal mammary nodes and in the absence of axillary LN metastasis

pN3a : Metastases in 10 or more axillary lymph nodes (at least one tumor deposit greater than 2.0mm);or metastases to the infraclavicular (level 3 axillary lymph nodes and in internal mammary lymph nodes) nodes

pN3b : Metastases in clinically apparent ipsilateral internal mammary lymph nodes in the presence of one or more positive axillary lymph nodes or metastasis in more than 3 axillary lymph nodes and in internal mammary lymph nodes with microscopic metastasis detected by sentinel lymph node dissection.

pN3c : metastasis in supraclavicular lymph nodes

Distant metastases (pM):

pM : categories correspond to M categories

MX : distant metastasis cannot be assessed

M0 : No distant metastasis

M1 : Distant detectable metastasis as histologically proven larger than 0.2mm

Table 2: AJCC staging with TNM categories and five year survival rates.

AJCC staging	TNM categories	Five year survival rates
STAGE 0	Tis N0 M0	100%
STAGE 1	T1 N0 M0	10%
STAGE 2A	T0 N1 M0	92%
	T1 N1 M0	
	T2 N0 M0	
STAGE 2B	T2 N1 M0	81%
	T3 N0 M0	
STAGE 3A	T0 N2 M0	67%
	T1 N2 M0	
	T2 N2 M0	
	T3 N0 M0	
	T3 N1 M0	
STAGE 3 B	T4 Any N M0	54%
	Any T N3 M0	
STAGE 4	Any T Any N M1	20%

Lymph node involvement: Most powerful independent prognostic tool. 10 year survival rate drops from 75% to 30-40 % when node negativity is compared with nodal positivity. The marginal cut off from which the prognosis gets bad is an involvement of 3 lymph nodes with secondary deposits. Worse prognosis if the internal mammary group of lymph nodes or higher level of axillary nodes show secondary carcinomatous deposit. This prognostic indicator has also been included in TNM staging and other prognostic index.

Some recent terminologies as regard with lymph node involvement include:

ITC: Isolated Tumor Cell clusters: Lymph node examination shows deposit less than 0.2mm would qualify as equivalent to node negative stage.

Micrometastasis: Lymph node examination shows deposit upto 2mm which is regarded as equivalent to node positivity. Studies have proved that a slight decrease in survival with micrometastasis.

Sentinel lymph node sampling: Gaining importance recently is the sentinel lymph node biopsy, but research and the results thus obtained have not given consistent results. But axillary dissection and sentinel lymph node sampling weigh almost equal in clinically node negative patients.

Extra capsular metastasis is a dependent variable on number of lymph nodes involved reflecting poor prognosis (98-108).

Histologic type: Certain histologic types have better prognosis than others. More favourable than invasive ductal carcinoma of No Specific Type are tubular carcinoma, mucinous carcinoma, invasive cribriform carcinoma, adenoid cystic carcinoma and invasive tubulolobular carcinoma. Grade correlated studies also show much better prognosis for Medullary carcinoma and classic variant of invasive lobular carcinoma (90).

Histologic grade: Most powerful prognostic indicator. The current widely accepted is the Nottingham's grading system- Elston Ellis modification of Scarff Bloom Richardson grading system. Regardless of morphological type, grading is advocated for all as it serves to prognosticate the metastasis and survival, independent of lymph node status. It also serves to predict response to chemotherapy (77, 90)

Nottingham's **Histological grading of breast carcinoma:**

Table 3: Elston-Ellis modification of Scarff Bloom Richardson Grading

Histological feature	Score 1	Score 2	Score 3
Tubule formation	>75% of tumor	10-75 % of tumor	<10 % of tumor
Nuclear pleomorphism	Minimal	Moderate	Marked
Mitosis / 10 Hpf (0.44mm field diameter/0.152mm^2 field area)	0-5	6-10	>11

Grade 1 includes Score 3-5

Grade 2 includes Score 6 and Score 7

Grade 3 includes Score 8 and Score 9

Lymphovascular invasion: Lymphatic invasion is a major prognostic factor with increased recurrence in node negative patients, possibly due to the occult metastasis. It is highly valuable in T1 node negative patients. It is more useful for assessing long term survival. Until recently only haematoxylin and eosin stained sections were used but more accurate invasion has now been possible with immunohistochemical staining.

Vascular invasion results have been variable nevertheless predicts local recurrence in conservative surgery and flap recurrence in mastectomy.[90]

Tumor cell infiltrate

Tumor cell infiltrates are seen associated with higher histological grade. Whether it is an independent prognostic tool is yet to be assessed with clarity.[77,90]

Extent of intra ductal component

The presence of intraductal component gains high importance in predicting recurrence in patients managed with conservative treatment and radiotherapy.[77,90]

Presence of necrosis

One of the feature when present, refers to earlier treatment failure and when extensive points to poor patient survival rates. Correlates with high grade and basal phenotype.[54]

Stromal features

The lime light is now on the behaviour of stroma with regards of invasion. Stromal fibrosis has shown inconsistent results related to prognosis, but fibrosis at center of lesion is an indicator of adverse prognosis. Elastosis occurring in the stroma seems to be associated with distinct histologic types namely tubular, tubular mixed and cribriform thus associated with good prognosis. Elastosis is a predictive marker reflecting lower response rate to hormone therapy. The absence of inflammation at periphery seems to be associated with less nodal positivity and good prognosis. High proportion of tumor cells to stroma carries a better prognosis.[54,77,90]

Combined morphologic prognostic factors: NPI is discussed in detail later.

ER: Estrogen receptor is a member of steroid receptor family. It governs gene expression for regulation of transcription and cell differentiation. Once estrogen binds with receptor there is dissociation of hsp 90 and hsp70 and dimerization occurs. ER dimers further interact with estrogen receptor elements and function to regulate transcription. Estrogen receptor alpha is the most widely studied which predicts response to treatment. Targeted therapy for ER has been used. ER negativity correlates well with higher proliferative index, poorly differentiated types.ER positivity predicts response to tamoxifen.

Grading of estrogen receptor positivity includes:

Number of tumor cell nuclei positive and intensity of staining.

ER negativity is associated with grade 3 histologic grade, carcinoma with pushing margins, lymphoid stroma, comedo necrosis, central fibrosis and necrosis.[77,90]

Medullary, metaplastic and apocrine are ER negative. Mucinous, tubular and lobular are ER positive. ER is less in premenopausal than post-menopausal patients. ER is associated with high nuclear grade and low histologic grade. Absence of tumor necrosis, marked elastosis are seen with older patients. Androgen receptor positivity is seen with lobular carcinoma, apocrine carcinoma, and Paget's disease and Estrogen negativity.

PR is an adjuvant marker predicting response along with ER: Both positive show 60-70 % response rate compared to 40 % with only ER and less than 10 % for both negative.[90]

HER 2/ neu (erbb2): helps to predict response and survival to some extent. Positivity associated with poor response to alkylating agents and favourable response to anthracyclines. Targeted therapy tried against her 2/neu with trastuzumab shows 20% response rate.[90]

Tp 53: Detected using DNA sequencing and showing mutations in l2 and l3 domain. Indicates poor prognosis. Even more is the loss of heterozygosity which predicts therapeutic response .[77]

Proliferation markers :[77]

Mitotic count

Assessment of mitotic count in Hematoxylin and Eosin stained histopathological section, forms a part of Nottingham's histopathological grading.

S phase fraction. High SPF indicates a poorer outcome. Assessed using DNA flow cytometry. Tumor heterogeneity and unstandardized values serve as disadvantages.

H thymidine labelling index: Used in fresh frozen section, determined by autoradiography. Measures cells undergoing DNA replication.

Thymidine kinase: Measured by radio enzymatic assay. High levels in G1-G2 phases. In breast carcinoma high levels of its fetal form are observed.

Ki 67 / MIB1: Measured using immunohistochemistry. It is a non histone nuclear protein. Labels cells in G1 to M phase indicating cell proliferation. Good prognostic marker helping to identify good and bad prognosis.

Cyclin: Group of proteins regulating cell cycle. Cyclin A detected during S, G2, M phase and Cyclin E1, Cyclin E2 in G1 phase and D1 in mid G1 phase all dictate poor prognosis.

P27: Cyclin dependent kinase inhibitor. Most frequently seen with ER positive tumors, low in BRCA 1 /2 associated tumors. Correlates in an inverse fashion with grade.

Topoisomerase II alpha: Cell cycle dependent enzyme with nuclease, helicase and ligase actions. Overexpression reflects good response to anthracyclines.

Urokinase plasminogen activator and urokinase plasminogen activator inhibitor: Serve as prognostic and predicative marker.

Other prognostic factors include:

The breast carcinoma involving medial half is associated with higher rates of relapse and death; Histopathologically breast carcinoma with pushing margins have better prognosis. The ones with skin invasion show lesser survival rates. Local recurrence shows bad prognosis.

Though individual prognostic factors give information about the survival rates, combination of these factors can give and achieve better level of accuracy about the survival of breast carcinoma patients.

Most widely accepted among them is the Nottingham's Prognostic index.[109]It includes three prognostic factors:

1. Tumor size
2. Lymph node stage
3. Histological grading

NPI= [size (cm) ×0.2] + lymph node stage (1-3) + Grade (1-3)

This was further divided into prognostic groups based on the score of NPI:[110,111]

Table 4: Prognostic groups based on NPI along with the ten year survival rate

Prognostic groups	NPI score	Ten year Survival rates
Excellent Prognostic Group(EPG)	2.08 to 2.4	95%
Good Prognostic Group(GPG)	>2.42 to <3.4	91%
Moderate Prognostic Group I(MPG I)	>3.42 to <4.4	80%
Moderate Prognostic Group II(MPG II)	>4.42 to <5.4	70%
Poor Prognostic Group(PPG)	>5.42 to < 6.4	45%
Very Poor Prognostic Group(VPG)	>6.5 to <6.8	33%

The most important factor for survival of breast carcinoma patient is invasion and metastasis. The tumor microenvironment plays a remarkable role. There are undiscovered molecular pathways and altered gene products which are either secreted by tumor cells themselves or made to secrete by the stromal cells under the influence of tumor cells. To one such group belongs the matrix metalloproteinase (MMP) family. MMP-2, a member of the family, is seen in association with higher oestrogen exposure and correlates well with

prognosis. Tissue inhibitors of MMPs are low in breast carcinoma with progesterone receptor expression. Also these MMP produce substances which act like chemotactic factors for tumor cells helping in migration.

CD 10

CD 10 Structure and function in normal tissues

It is a 90-110 kilo Dalton cell surface, membrane bound, zinc dependent matrix metalloproteinase. Most commonly known as Common Acute Lymphoblastic Leukemia Antigen (CALLA) and is also known as enkephalinase in the brain, membrane metalloprotease, skin fibroblast elastase .It bears similarity with stromelysin, a matrix metalloproteinase. CD 10 codes for protein **Neprilysin**. It is a glycoprotein present in the brush border of proximal convoluted tubules and on the glomerular epithelium. It is a neutral endopeptidase that cleaves peptides at the amino side of hydrophobic residues and inactivates several peptide hormones including glucagon, enkephalins, substance P, neurotensin, oxytocin, and bradykinin. This gene, which encodes type II transmembrane glycoprotein, exists in a single copy of greater than 45 kilobases. The 5' untranslated region of this gene is alternatively spliced, resulting in separate mRNA transcripts.

In the bone marrow it stimulates differentiation of Pre B cells and myeloblasts helping in cell proliferation and motility.

CD 10 in normal breast tissue

In normal breast tissue, CD10 maintains the progenitor pool of stem cells by cleaving signalling proteins and growth factors, thereby the stem cells remain in the pool and are not differentiated to form luminal or myoepithelial cells. This is facilitated by integrin beta 1. CD 10 is also expressed by the myoepithelial cells of the breast but gets lost with the onset of invasive cancer as the source cell (myoepithelial) disappears in carcinoma.

CD10 in invasive breast carcinoma

Interestingly in invasive carcinoma, there are numerous genetic alterations, which trigger either myofibroblasts or through conversion of fibroblasts to myofibroblasts or stromal cells to expresses and secrete CD 10 extracellularly, making way for cleavage of protein components of extracellular matrix. As a consequence, the matrix metalloproteinase acts to alter the stromal microenvironment, making it easier to be invaded by carcinomatous cells. Though some studies reveal that the exact cell secreting CD 10 is not known, CD10 is involved in cell proliferation, angiogenesis, increasing cell locomotion, and in making cells resistant to apoptosis. During carcinogenesis, the tissue microenvironment undergoes extensive remodelling, including changes in deposition, degradation, and structural organization that involve all tissue components including stem cells. This

remodelling involves many enzymes that control cell survival, proliferation, migration, polarization, and differentiation.

The impact of stroma can be high as it paves way for angiogenesis through which cells can migrate after detachment and metastasize, increasing the grade of the tumor and thereby decreasing the survival rate of patients.

CD 10 is currently undergoing molecular and structural research, enabling a more understanding of molecular alterations and drug designing for target therapies. CD 10 has been claimed to have 2 different properties, one is the enzymatic activity and the other is a signalling activity. Enzymatic activity has been elaborated in normal breast tissue functions.

In carcinoma, the mutations in stem cells decrease CD 10 causing accumulation of unprocessed peptides leading onto lineage commitment of stem cells and higher proliferating potential. Another proposed pathway involves mutated CD10 activity mostly involving the mesenchymal stem cells leading to accumulation of cleaved peptides inhibiting stem cell differentiation and maintaining undifferentiated pool leading to undifferentiated carcinomas. Of these, whichever pathway plays a role, it favours proliferation of stem cell pool and promote carcinogenesis. TGF beta causes increase in stromal expression of CD10.

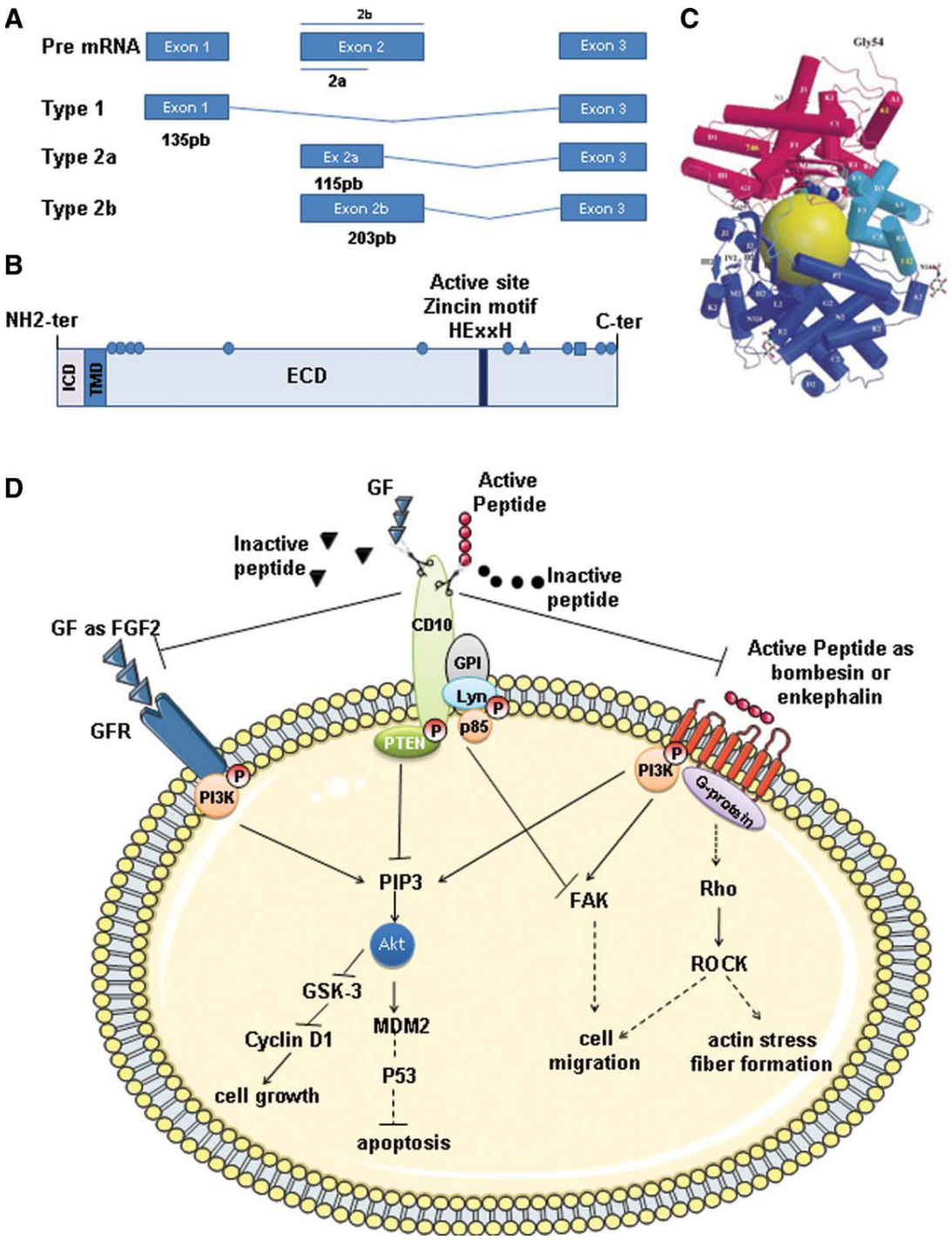


Figure 1: CD10 molecule.

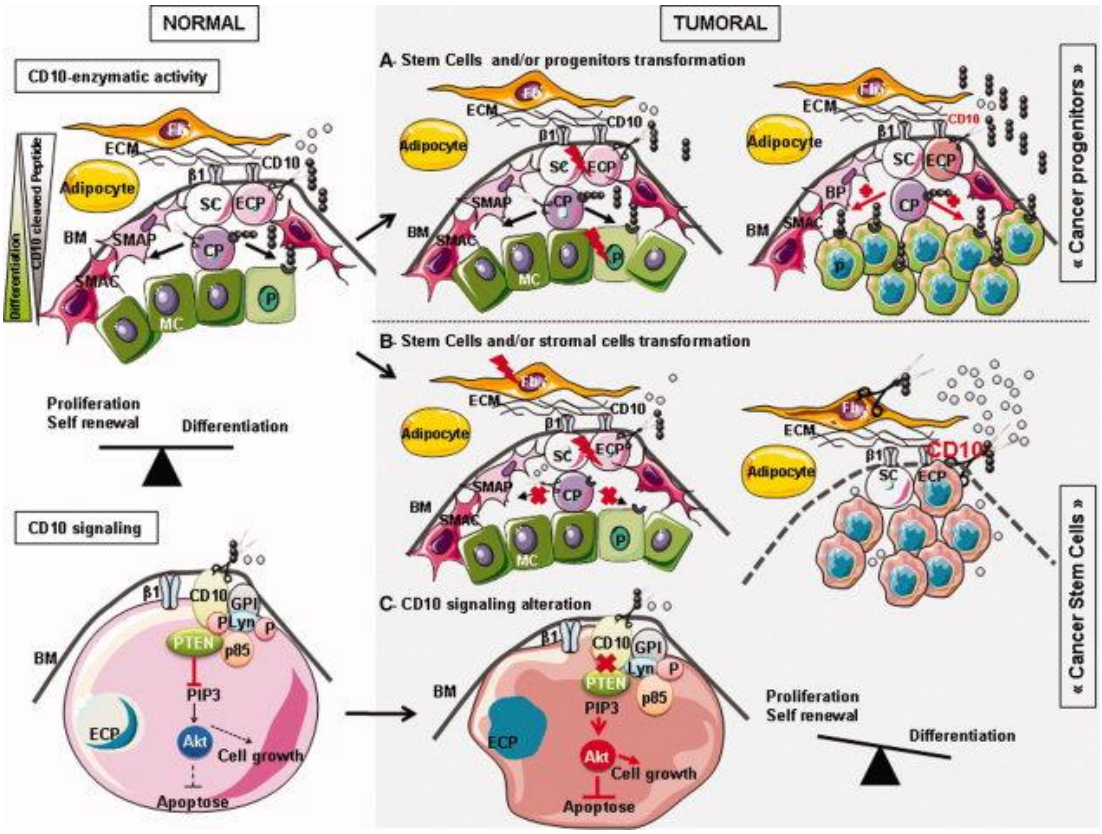


Figure 2: CD 10 Mechanism of action.

The other signalling pathway involves PTEN. PTEN and CD 10 act combined to decrease PIP3 which is involved in stimulating Akt. Akt inhibits apoptosis and induces cell growth (MDM2 pathway). CD 10 also acts to cleave FGF which acts through Akt pathway to induce endothelial cell growth and angiogenesis. CD 10 is also involved in cell migration. This explains the mitotic grade of such tumors.[112]

CD 10 and CHEMOTHERAPY: The CD 10 signalling pathway is also claimed to be responsible for chemotherapy induced resistance.

CD 10 cleaves the chemotherapeutic agent doxorubicin increasing the resistance to chemotherapy. Thus drugs which can target CD 10 can also decrease chemo resistance thereby increasing the sensitivity of patients to chemotherapy. Also the property of CD 10 molecule in cleaving prodrugs of doxorubicin namely CPI-0004Na [N-succinyl-β-alanyl-L-leucyl-Dox (sALAL-Dox)] is widely investigated which on success can bring down the dose and dosage related side effects of doxorubicin.

Recent advancements have enumerated more stromal alterations describing 2 types of stroma in breast carcinoma. One is solitary fibrous type and the other is desmoid type. Of the two, solitary fibrous type stroma is associated with poorer prognosis. The desmoid type of stroma has better prognosis but when associated with CD 10 expression shows poor prognosis, implicating that CD 10 is an independent tumor prognostic marker. These researches have thrown light on the field of tissue remodelling. Extracellular matrix degradation is associated with tissue remodelling.CD 10 is not expressed by normal breast stromal cells, fibroblasts or adipose tissue. CD 10 is also expressed by proliferating stromal cell as in phylloides.

Recent researches are on identification of molecules that targeted against CD 10 molecule which can help in controlling invasion and progression of low grade carcinomas.

Following studies have thrown light on some aspects of CD10

Keiichi Iwaya et al (2002) studied the stromal expression of CD10 in 123 cases of carcinoma breast, of them 13 were non-invasive and 110 were invasive breast carcinoma. 20 out of 110 invasive breast carcinoma cases were CD10 positive and all non-invasive cases were CD10 negative. Positive statistical significance was seen in axillary lymph node metastasis. It was also statistically proved to predict time for recurrence and prognostic factor indicating survival.[4]

Nikita A Makretsov et al (2007) studied the stromal expression CD10 in invasive breast carcinoma with 258 cases of invasive carcinoma cases and 15 cases of DCIS. CD10 expression showed significant correlation with ER negativity, high tumor grade and decreased survival. Tissue microarray was the methodology employed.[5]

Vandana Puri et al (2011) studied the expression of CD10 in 50 patients with breast carcinoma out of which 40 were positive and found that it correlated strongly with HER2neu and Ki67 positivity, ER/PR negativity and higher tumor grade .[113]

Fereshteh Mohammadizadeh et al (2012) had studied the stromal expression of CD10 in invasive breast carcinoma in 49 cases and found significant correlation of CD10 expression with tumor size, axillary lymph node status and tumor grade.[114]

Hala N. Hosni et al (2012) had studied CD10 stromal expression in 50 mammary insitu and invasive duct carcinoma and found significant correlation of CD10 expression with tumor grade.[115]

Sayantan H.Jana et al (2014) had studied the expression of CD10 in 70 cases of breast cancer (69 cases of radical mastectomy and 1 case of trucut biopsy) and found statistically significant association of CD10 stromal expression with increasing mitotic rate and tumor grade, worsening prognosis, ER negativity, HER2neu positivity. Significant association with molecular subtypes namely CD10 positivity with HER2 type and CD10 negativity with luminal type were also noted.[116]

Ali Taghizadeh-Kermani et al (2014) had studied the stromal expression of CD10 in 50 patients of fibroadenoma and 100 patients of invasive breast carcinoma. 28 percent immunoreactivity was observed in invasive ductal carcinoma and concluded that CD10 stromal expression significantly correlated with histological grade, increasing tumor size, presence of nodal metastases and ER negativity .[117]

Maha E. Salama et al (2015) had studied the stromal expression of CD10 in desmoplastic stroma of breast carcinoma and stroma of phylloides tumor. 36 cases of invasive ductal carcinoma and 34 cases of phylloides tumor were studied. CD 10 immunoreactivity seen in 77.8% cases of invasive ductal carcinoma and 32.4% cases of phylloides tumor. It was concluded that

high level of CD10 expression was correlated with malignant phylloides and high tumor grade breast carcinoma.[118]

B.V. Anuradha Devi et al (2016) had studied the stromal CD 10 expression in 59 cases of breast cancer and found out significant relationship of CD 10 with increasing tumor size, increasing tumor grade, worsening prognosis and lymph node status.[119]

Thomas S. et al (2013) studied the effect of neo-adjuvant chemotherapy on stromal CD10 antigens in breast cancer. 29 patients scheduled for chemotherapy were studied, 16 had strong CD10 expression. In these 16 patients, 14 were negative for hormonal receptors and 14 had HER2neu over expression. post chemotherapy studies remained same in 13 cases, decreased in 13 cases and increased in 3 cases. Out of 13 cases where CD10 expression was decreased, 12 had clinical response. So it was concluded that CD10 stromal expression correlated with hormone receptor negativity and HER2/neu over expression.[120]

IMMUNOHISTOCHEMISTRY

Immunohistochemistry was started in 1940 when Coons developed an immunofluorescence technique to detect corresponding antigen in frozen sections.

Taylor and colleagues in 1974 showed it was possible to demonstrate antigens in routinely processed tissue. Antigen retrieval technique was introduced by Shi and his associates in 1991.

MATERIALS AND METHODS

Study Design: Cross sectional study

METHODOLOGY

This study was done during the time period from June 2014 to June 2016. All patients who have undergone modified radical mastectomy and confirmed with the diagnosis of invasive ductal carcinoma by histopathological examination were included in the study population**.**

The institutional ethical committee approval was obtained**.**

Inclusion criteria: All cases reported as positive for invasive ductal carcinoma of breast, with first line of treatment as surgery were included in the study population.

Exclusion criteria: All cases who have undergone any other modalities of treatment including radiotherapy, chemotherapy or hormone therapy prior to surgery are excluded in view that there can be differences or alterations in the expression of the marker of interest.

Consent: may not be required as only tissues sent for histopathological examination or blocks of tissue are to be used.

Study Period: From June 2014 to June 2016.

Sample size: Consecutive 60 patients with histologically confirmed diagnosis of invasive ductal carcinoma of breast were chosen. The details of patient including the age, disease laterality, menopausal status, tumor size,

histopathological grade, lymph node metastasis and treatment details were obtained and data was entered in DATA entry form, sample of which has been attached at ANNEXURE II.

The blocks of these patient were retrieved and Haematoxylin and Eosin stained section was prepared and evaluated.

Immunohistochemistry procedure

Fixation: The tissue were fixed in 10 % neutral buffered formalin overnight.

Tissue processing and section cutting: The sections thus obtained were dehydrated using graded alcohol followed by xylol in an automatic histokinete. The sections were then impregnated and embedded in paraffin and blocks were obtained. 3 microns thin sections were cut using semi-automatic microtome. Poly L lysine coated slides were used.

Incubation: The sections were left in incubator for 1 hour at 60-70° C**.**

Deparaffinization and hydration: The sections were deparaffinized in 2 changes of xylene each lasting for 15 minutes. They were then hydrated through graded alcohol namely:

2 changes of absolute alcohol for 5 minutes each.

90 % alcohol for 5 minutes.

70% alcohol for 5 min.

Then the sections were washed in two changes of distilled water for 2 minutes each.

Antigen retrieval: The antigen retrieval was done using the Heat Induced Antigen Retrieval method (pressure cooker). The sections were immersed in TRIS EDTA buffer at PH 9.0 and left inside the pressure cooker for 40 minutes.

They were then washed with two changes of distilled water lasting for 2 minutes each. This was followed by washing in Tris buffer saline of PH 7.2

Blocking of endogenous peroxidase: Following this, addition of H2O2 was done for 5 minutes to block reaction from endogenous peroxidase. This was followed by 2 washes in TRIS buffer lasting for 2 minutes each.

Primary antibody: Primary antibody was then added and kept for 30 minutes. We had used CD10 mouse monoclonal antibody (Pathnsitu pm150).This was followed by 2 washes of buffer lasting for 2 minutes each.

Addition of target binder (secondary antibody) and HRP: The polyexcel target binder reagent was added for 12 minutes and washed in 2 changes of buffer. This was followed by addition of polyexcel HRP for 12 minutes followed by 2 buffer washes.

Addition of colouring agent: DAB Chromogen working solution was prepared by mixing 1ml DAB buffer with 1 drop of DAB chromogen. This was added to slide and kept for 5minutes and then washed with 2 washes of distilled water.

Counterstaining and mounting: The slides were then immersed in Haematoxylin (counterstain) for 3 minutes, washed with water and dehydrated in graded alcohols namely 70%, 90% and absolute alcohol followed by xylol clearing and finally mounting was done.

Positive control: We used section of fibroadenoma where the myoepithelial cells show strong immunoreactivity for CD 10 expression.

Negative control: We had used Phosphate buffer instead of Primary antibody.

Scoring was done as below:

Table 5: CD 10 expression interpretation

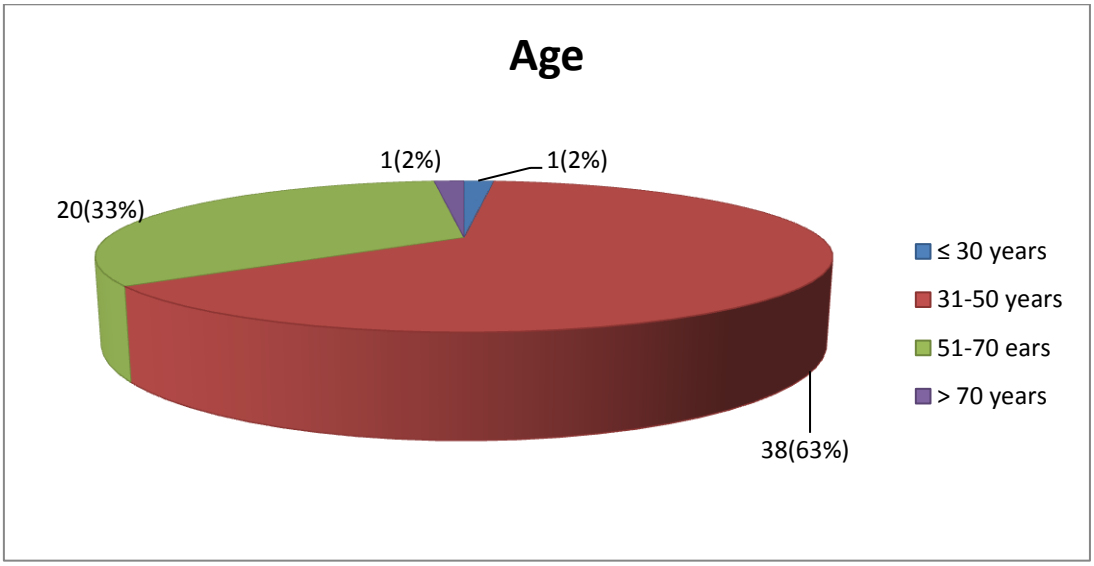
Interpretation	Description
Negative (Figure 13, 14)	No staining <10 % cytoplasmic and membranous staining in stromal cells
Weak positive (Figure 15,16)	10-30 % focal cytoplasmic and membranous staining of stromal cells Diffuse weak staining
Strong positive (Figure 17,18)	>30 % cytoplasmic and membranous staining of stromal cells.

The slides were interpreted and the collected data was entered into Excel sheet and statistically analysed using SPSS software version 16.0.

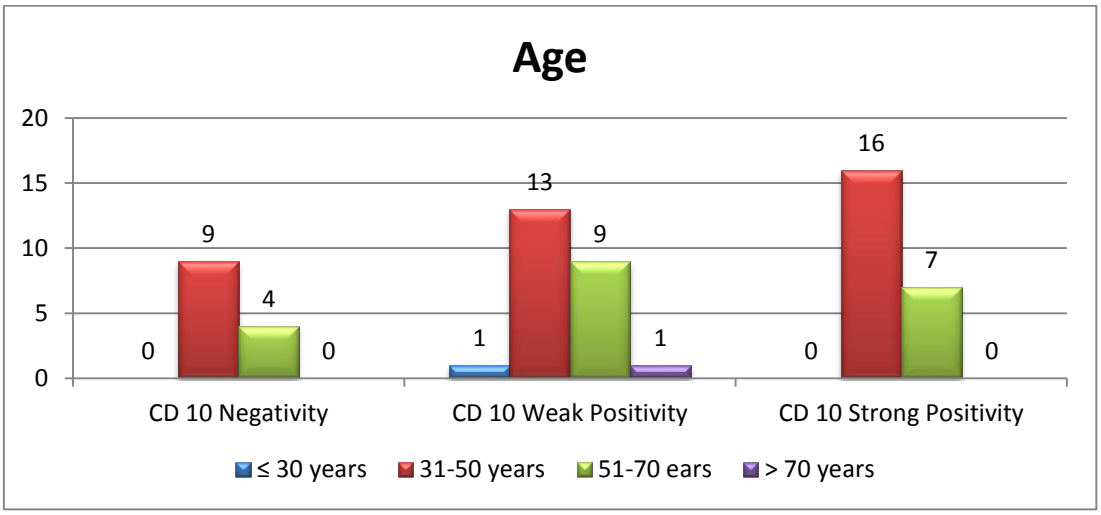
OBSERVATIONS AND RESULTS

The present study included a sample of 60 patients diagnosed as infiltrating ductal carcinoma of breast fulfilling the inclusion criteria. All of them had undergone modified radical mastectomy as first line of treatment. The data was analysed for the following parameters namely Age, laterality, menopausal status, histopathological variants, tumor size, positive lymph nodes, mitotic rate, lymph node grade, histopathological grade and NPI prognostic groups, statistically and represented in pie charts, bar diagrams and tables below.

AGE:



Graph 1: Age wise distribution of study sample



Graph 2: Comparison of CD 10 expression with age

Table 6: Age wise distribution of cases compared with expression of CD10

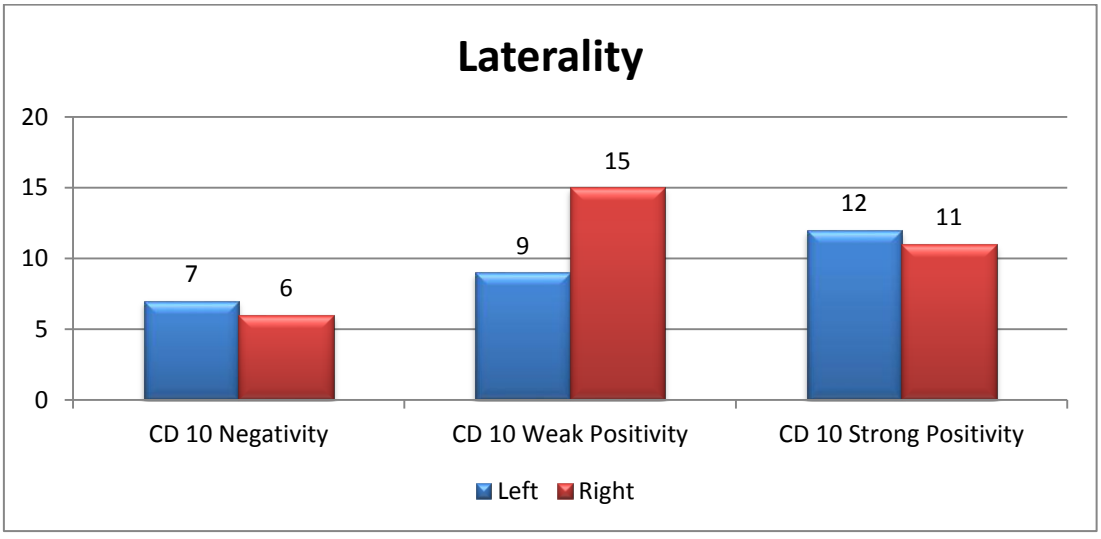
Age	Number of patients	CD 10 Negativity	%	CD 10 Weak Positivity	%	CD 10 Strong Positivity	%
≤ 30 years	1	0	0.00	1	4.17	0	0.00
31-50 years	38	9	69.23	13	54.17	16	69.57
51-70 ears	20	4	30.77	9	37.50	7	30.43
> 70 years	1	0	0.00	1	4.17	0	0.00
Total	60	13	100	24	100	23	100

Table 7: P value for comparison of CD expression with age

Age Distribution	CD 10 Negativity	CD 10 Weak Positivity	CD 10 Strong Positivity
Mean	46.15	50.71	49.52
Standard Deviation	9.63	13.51	10.04
P value (Single Factor ANOVA)			0.5149

The present study includes a sample of 60 patients with 1 patient less than 30 years of age showing weak positivity of CD 10 expression, 1 patient with more than 70 years of age showing weak positivity, among 20 patients between age groups of 51 to 70 years 4 of them were negative, 9 were weakly positive and 7 were strongly positive for CD10 expression. Maximum number of patients namely 38 patients were between age group 31 to 50 years. Out of them 9 were negative, 13 were weakly positive and 16 were strongly positive for CD 10 expression. Statistical analysis using single factor ANOVA was not significant P value (0.51) (Table 7).

Laterality



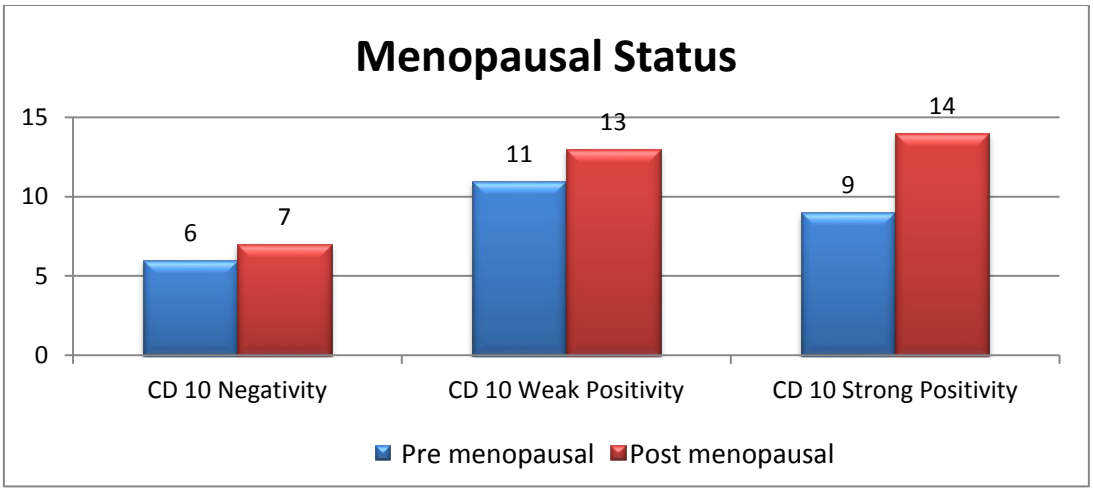
Graph 3: Comparison of CD10 expression with side of breast carcinoma

Table 8: P value for comparison of CD 10 expression with side of breast carcinoma

Side	Number of patients	CD 10 Negativity	%	CD 10 Weak Positivity	%	CD 10 Strong Positivity	%
Left	28	7	53.85	9	37.50	12	52.17
Right	32	6	46.15	15	62.50	11	47.83
Total	60	13	100	24	100	23	100
P value (Fishers Exact Test)					0.5658		

Out of the 60 patients, 28 of them had left breast involvement, out of which 7 were showing negative, 9 were weak positivity and 12 strong positivity for CD10 expression. 32 patients had right breast involvement out of which 6 of them were negative, 15 weakly positive and 11 strongly positive for CD 10 expression. Statistical analysis found out the association was not significant with P value (0.56) (table 8).

Menopausal status



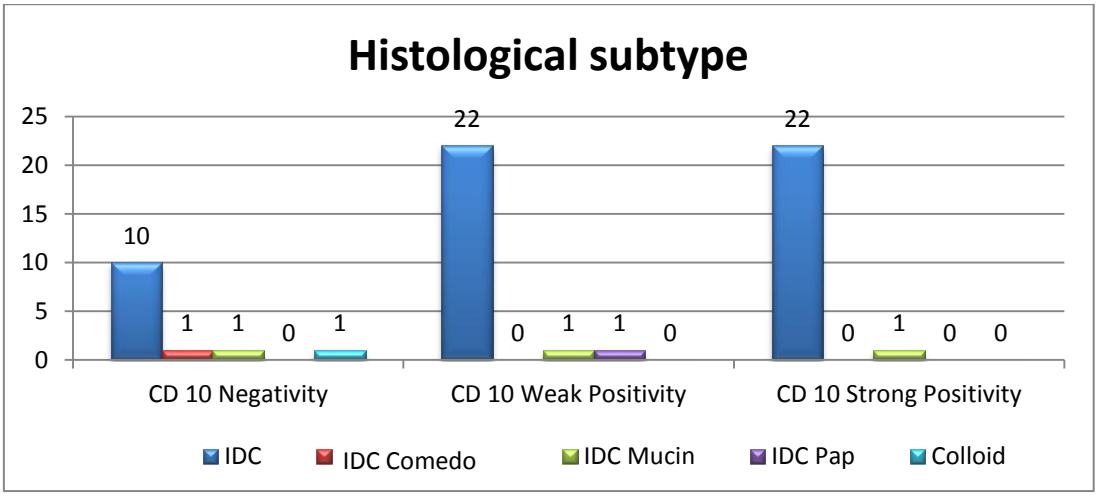
Graph 4: Comparison CD 10 expression with menopausal status

Table 9: P value for Comparison CD 10 expression with menopausal status

Menopausal Status	Number of patients	CD 10 Negativity	%	CD 10 Weak Positivity	%	CD 10 Strong Positivity	%
Pre-menopausal	26	6	46.15	11	45.83	9	39.13
Post-menopausal	34	7	53.85	13	54.17	14	60.87
Total	60	13	100	24	100	23	100
P value (Fishers Exact Test)					0.8923		

Out of the 60 patients, 26 of them were in pre-menopausal/ reproductive age group among who 6 were negative, 11 were weakly positive and 9 were strongly positive for CD 10 expression. 34 patients were post-menopausal. Of them 7 were negative, 13 were weakly positive and 14 were strongly positive. Statistical analysis showed a P value of 0.89 indicating no significant statistical association (Table9).

Histological Subtype of Invasive ductal carcinoma



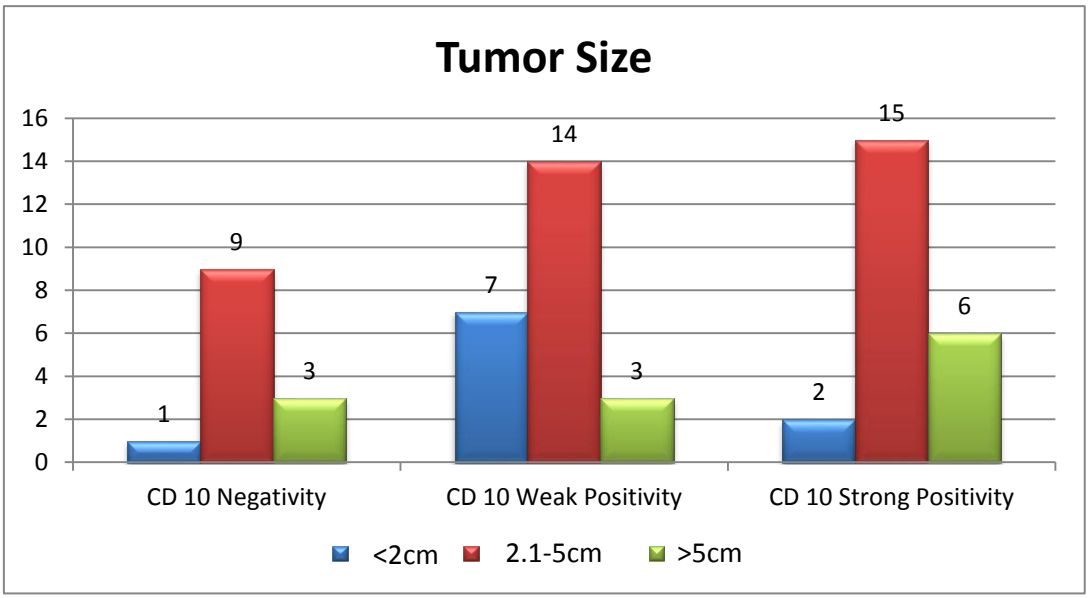
Graph 5: Comparison of CD 10 expression with histological subtypes of invasive ductal carcinoma
(IDC Mucin- IDC with mucinous differentiation; IDC Pap-IDC with papillary differentiation; Colloid-Colloid carcinoma)

Table 10: P value for comparison of CD 10 expression with histological subtypes of invasive ductal carcinoma

Diagnosis	Total number of patients	CD 10 Negativity	%	CD 10 Weak Positivity	%	CD 10 Strong Positivity	%
IDC NST	54	10	76.92	22	91.67	22	95.65
IDC with Comedonecrosis	1	1	7.69	0	0.00	0	0.00
IDC with Mucin	3	1	7.69	1	4.17	1	4.35
IDC with Pap	1	0	0.00	1	4.17	0	0.00
Colloid	1	1	7.69	0	0.00	0	0.00
Total	60	13	100	24	100	23	100
P value Fishers Exact Test					0.5284		

Out of 60 patients, 54 of them were diagnosed as invasive ductal carcinoma no specific type (IDC NST) among them 10 were negative, 22 were weakly positive and 22 were strongly positive for CD10 expression. 1 patient with diagnosis of colloid carcinoma and 1 with IDC with comedo pattern, both of them were CD 10 negative.3 patients were with IDC showing mucinous differentiation of whom, 1 was negative, 1 was weak positive and 1 strong positive. 1 patient with IDC with papillary areas was weakly positive for CD10 expression. Statistical analysis for their association showed no significance with P value 0.52 (Table 10).

Tumor size



Graph 6: Comparison of CD 10 expression with tumor size

Table 11: Comparison of CD 10 expression with tumor size

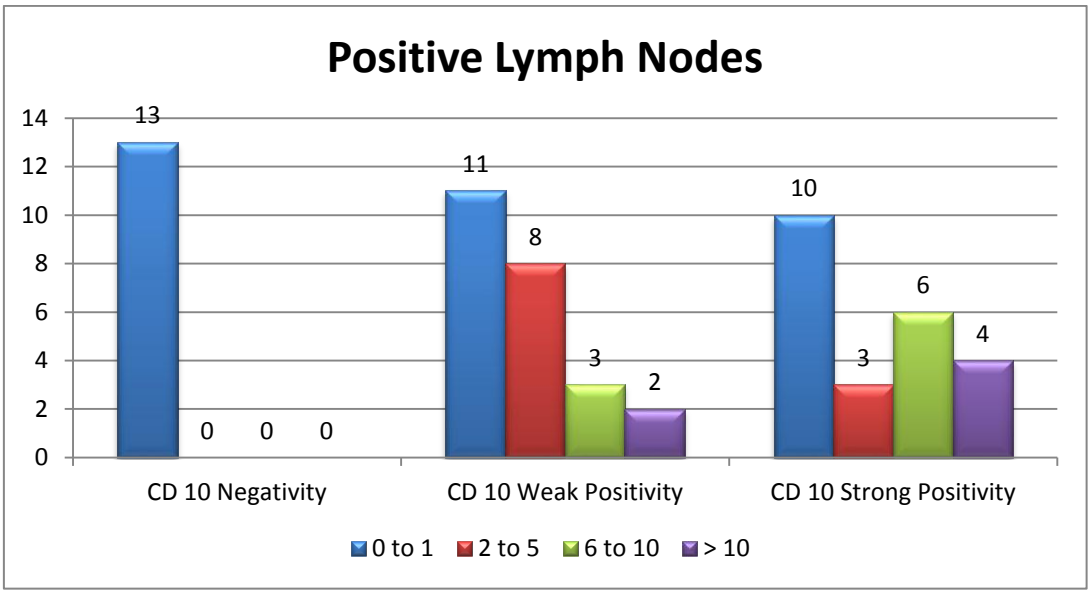
Tumor Size	Number of patients	CD 10 Negativity	%	CD 10 Weak Positivity	%	CD 10 Strong Positivity	%
≤ 2 cm	10	1	7.69	7	29.17	2	8.70
2.1 - 5.0 cm	38	9	69.23	14	58.33	15	65.22
>5.0 cm	12	3	23.08	3	12.50	6	26.09
Total	60	13	100	24	100	23	100

Table 12: P value for comparison of CD 10 expression with tumor size

Tumor Size Distribution	CD 10 Negativity	CD 10 Weak Positivity	CD 10 Strong Positivity
Mean	4.58	3.66	4.59
Standard Deviation	2.14	1.99	2.64
P value (Single Factor ANOVA)			0.3192

Out of 60 patients, 38 patients had a tumor size 2 to 5cm of whom 9 were negative, 14 were weakly positive and 15 were strongly positive for CD10 expression. 10 patients had tumor size less than 2cm among them 1 was negative, 7 were weakly positive and 2 were strongly positive for CD 10 expression. 12 patients had tumor size more than 5 cm, among them 3 were negative, 3 were weakly positive and 6 were strongly positive for CD 10 expression. On statistical analysis using single factor ANOVA the association was not found to be statistically significant, with p value of 0.31 (Table11,12)

Positive Lymph Nodes



Graph 7: Comparison of CD 10 expression with lymph node positivity on histopathological examination

Table 13: Comparison of CD 10 expression with lymph node involved on histopathological examination

Positive Lymph Nodes	Total number of patients	CD 10 Negativity	%	CD 10 Weak Positivity	%	CD 10 Strong Positivity	%
0 to 1	34	13	100.00	11	45.83	10	43.48
2 to 5	11	0	0.00	8	33.33	3	13.04
6 to 10	9	0	0.00	3	12.50	6	26.09
> 10	6	0	0.00	2	8.33	4	17.39
Total	60	13	100	24	100	23	100

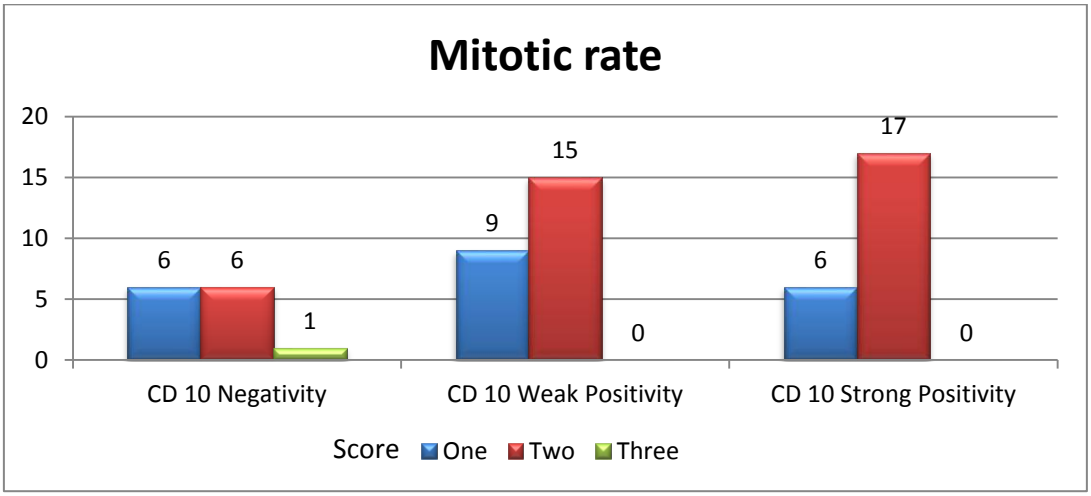
Table 14: P value for comparison of CD 10 expression with lymph node positivity on histopathological examination

Positive Lymph Nodes Distribution	CD 10 Negativity	CD 10 Weak Positivity	CD 10 Strong Positivity
Mean	0.08	3.50	4.74
SD	0.28	4.80	5.02
P value Single Factor ANOVA			0.0017

Out of the 60 patients, 34 patients had from 0 to 1 lymph node involvement of who 13 were negative, 11 were weakly positive and 10 were strongly positive for CD 10 expression. 11 patients had 2 to 5 lymph nodes involved, of them 8 were weakly positive and 3 were strongly positive for CD 10 expression. 9 patients had 6 to 10 lymph nodes, of them 3 were weakly positive and 6 were strongly positive for CD 10 expression. 6 patients had more than 10 lymph node involved of them 2 were weakly positive and 4 were strongly positive. Statistical analysis was done for comparison of CD 10

expression with positive lymph nodes involved by tumor, P value 0.0117 was obtained and found to be statically significant (Table 14).

Mitotic rate [77]



Graph 8: Comparison of CD10 with mitotic rate

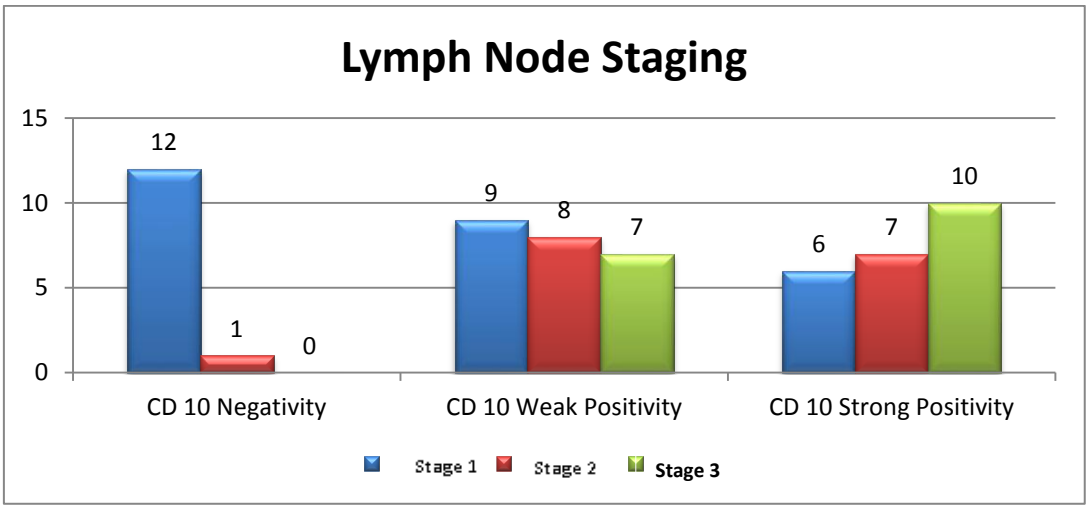
Table 15: P value for comparison of CD 10 expression with mitotic rate

Mitotic Rate	Total number of patients	CD 10 Negativity	%	CD 10 Weak Positivity	%	CD 10 Strong Positivity	%
Score One	21	6	46.15	9	37.50	6	26.09
Score Two	38	6	46.15	15	62.50	17	73.91
Score Three	1	1	7.69	0	0.00	0	0.00
Total	60	13	100	24	100	23	100
	P value Fishers Exact Test				0.4449		

Out of the 60 patients, 21 patients had mitotic score 1 of whom, 6 were negative, 9 weakly positive and 6 strongly positive for CD 10 expression. 38 patients had mitotic score 2, 6 of them negative, 15 weakly positive and 17

strongly positive for CD 10 expression. Only one patient had mitotic rate 3 and was negative for CD 10 expression. P value by fisher exact test was 0.449 and thus the correlation between CD10 expression and mitotic rate was statistically not significant (Table 15).

Lymph node stage [90]



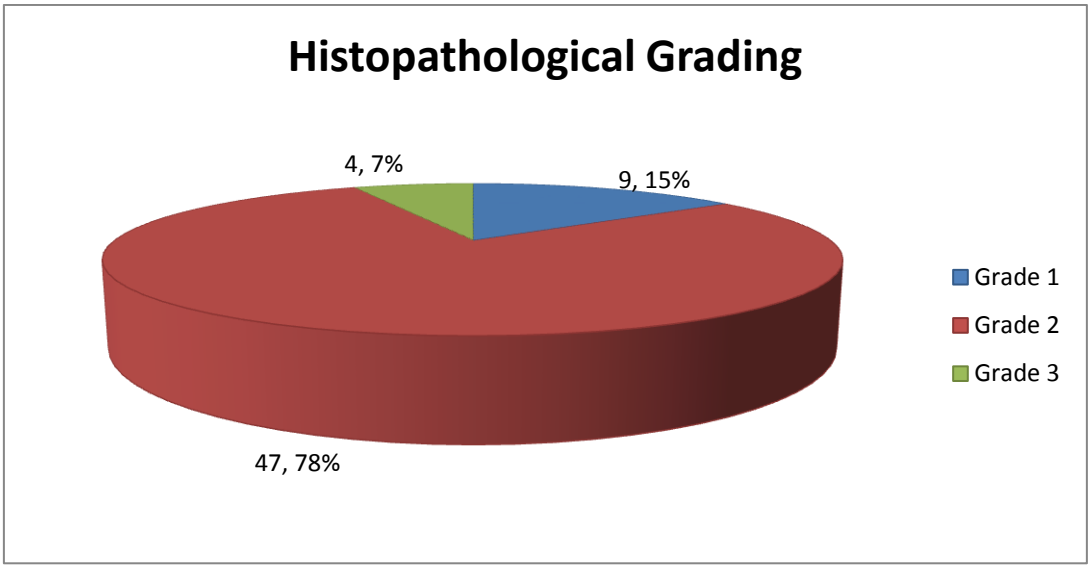
Graph 9: Comparison of CD 10 expression with lymph node staging

Table 16: P value for comparison of CD 10 expression with lymph node staging

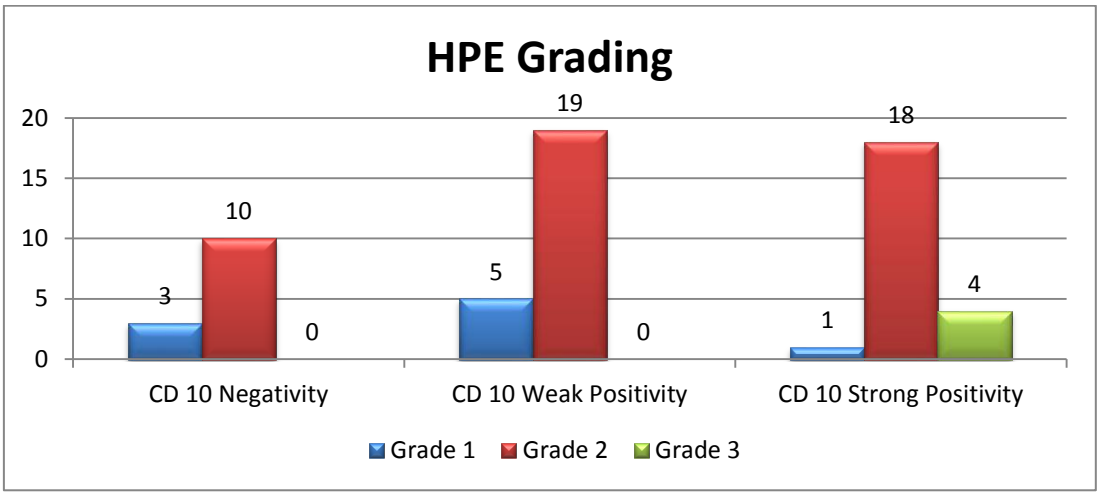
Lymph Node Staging	Number of patients	CD 10 Negativity	%	CD 10 Weak Positivity	%	CD 10 Strong Positivity	%
Stage 1	27	12	92.31	9	37.50	6	26.09
Stage 2	16	1	7.69	8	33.33	7	30.43
Stage 3	17	0	0.00	7	29.17	10	43.48
Total	60	13	100	24	100	23	100
P value Fishers Exact Test					0.0136		

Out of 60 patients, 27 patients had lymph node stage 1, 12 of whom were negative, 9 were weakly positive and 6 were strongly positive for CD 10 expression. 16 patients were of lymph node stage 2, 1 was negative, 8 were weakly positive and 7 were strongly positive for CD 10 expression. 17 patients were of lymph node stage 3, with 7 of them weakly positive and 10 of them strongly positive for CD 10 expression. P value obtained by fisher exact test was 0.0136 and found to be statistically significant (Table 16).

Histopathological Grade [77]



Graph 10: Histopathological grade wise distribution of study sample



Graph 11: Comparison of CD 10 expression with Histopathological grade

Table 17: P value for comparison CD 10 expression with histopathological grade

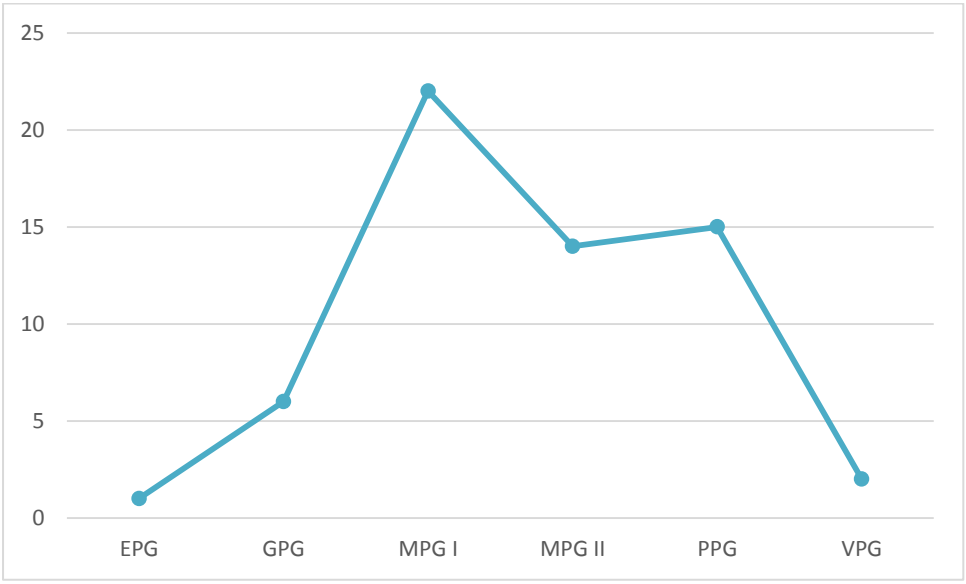
HPE Grading	Number of patients	CD 10 Negativity	%	CD 10 Weak Positivity	%	CD 10 Strong Positivity	%
Grade 1	9	3	23.08	5	20.83	1	4.35
Grade 2	47	10	76.92	19	79.17	18	78.26
Grade 3	4	0	0.00	0	0.00	4	17.39
Total	60	13	100	24	100	23	100
P value Fishers Exact Test					0.0472		

Out of 60 patients, 9 patients were of histopathological grade 1, of whom 3 were negative, 5 were weakly positive and 1 was strongly positive for CD 10 expression. Maximum number of patients were of grade 2, namely 47 patients of whom 10 were negative, 19 were weakly positive and 18 were strongly positive for CD 10 expression. 4 of them were of grade 3, all showed

strong positive immunoreactivity with CD 10. P value obtained by Fishers exact test was 0.0472 and thus statistically significant (Table 17).

Nottingham's Prognostic index

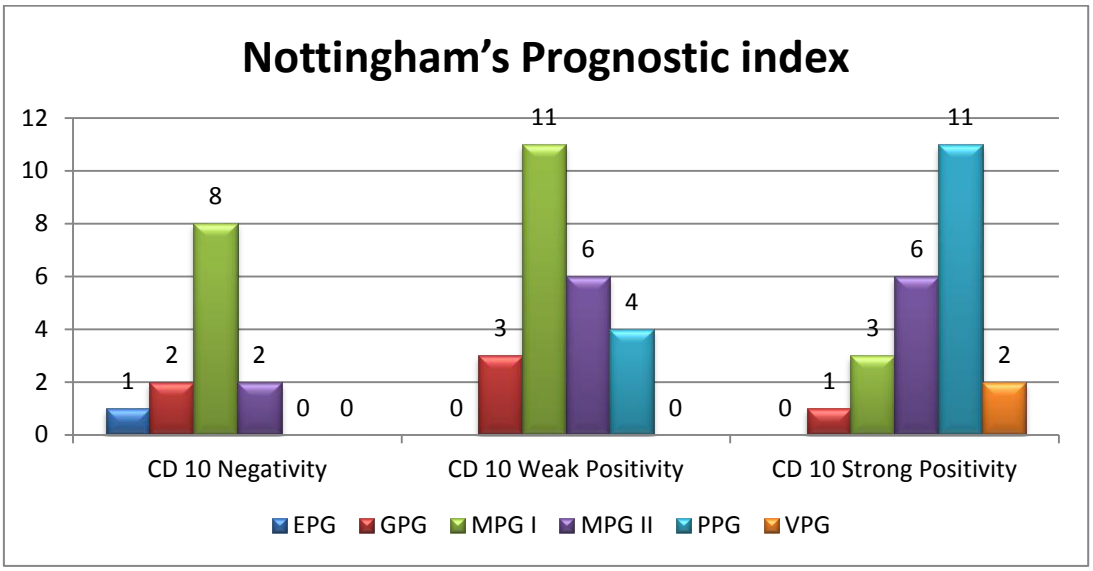
The following is the distribution of our patients divided into prognostic groups



Graph 12 : Distribution of study sample based NPI prognostic groups

Table 18 : NPI prognostic group based distribution of study sample

Prognostic groups based on NPI	No of patients
Excellent prognostic group (EPG)	1
Good prognostic group (GPG)	6
Moderate prognostic group I (MPG I)	22
Moderate prognostic group II (MPG II)	14
Poor prognostic group (PPG)	15
Very poor prognostic group (VPG)	2



Graph 13: Comparison of CD 10 expression with prognostic groups based on NPI

Table 19: P value for comparison of CD 10 expression with prognostic groups based on NPI

Nottingham's Prognostic index	Number of patients	CD 10 Negativity	%	CD 10 Weak Positivity	%	CD 10 Strong Positivity	%
EPG	1	1	7.69	0	0.00	0	0.00
GPG	6	2	15.38	3	12.50	1	4.35
MPG I	22	8	61.54	11	45.83	3	13.04
MPG II	14	2	15.38	6	25.00	6	26.09
PPG	15	0	0.00	4	16.67	11	47.83
VPG	2	0	0.00	0	0.00	2	8.70
Total	60	13	100	24	100	23	100
P value Fishers Exact Test					0.0092		

Out of 60 patients, 1 belonged to Excellent Prognostic Group, and was negative for CD10 expression. 6 patients were Good Prognostic Group, of whom 2 were negative, 3 were weakly positive and 1 was strongly positive for Cd 10 expression. 22 patients were Moderate Prognostic Group I, of whom 8 were negative, 11 were weakly positive and 3 were strongly positive for CD 10 expression. 14 patients were Moderate Prognostic Group II, among them 2 were negative, 6 were weakly positive and 6 were strongly positive for CD 10 expression. 15 patients belonged to Poor Prognostic Group, of them 4 were weakly positive and 11 were strongly positive for Cd 10 expression. Only 2 patients were of Very Poor Group, and was strongly positive for CD 10 expression. P value obtained by Fisher exact test was 0.0092 and statistically significant (Table 19).

Fig.3: Gross: Invasive Ductal Carcinoma – NST

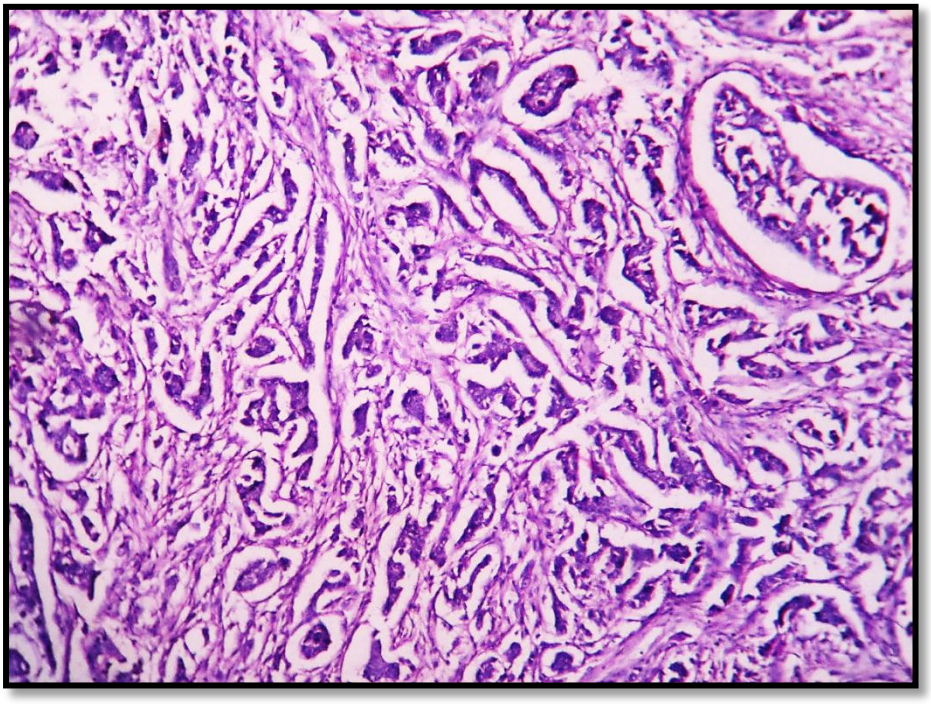


Fig.4: H & E, IDC grade 1, 200 X magnification

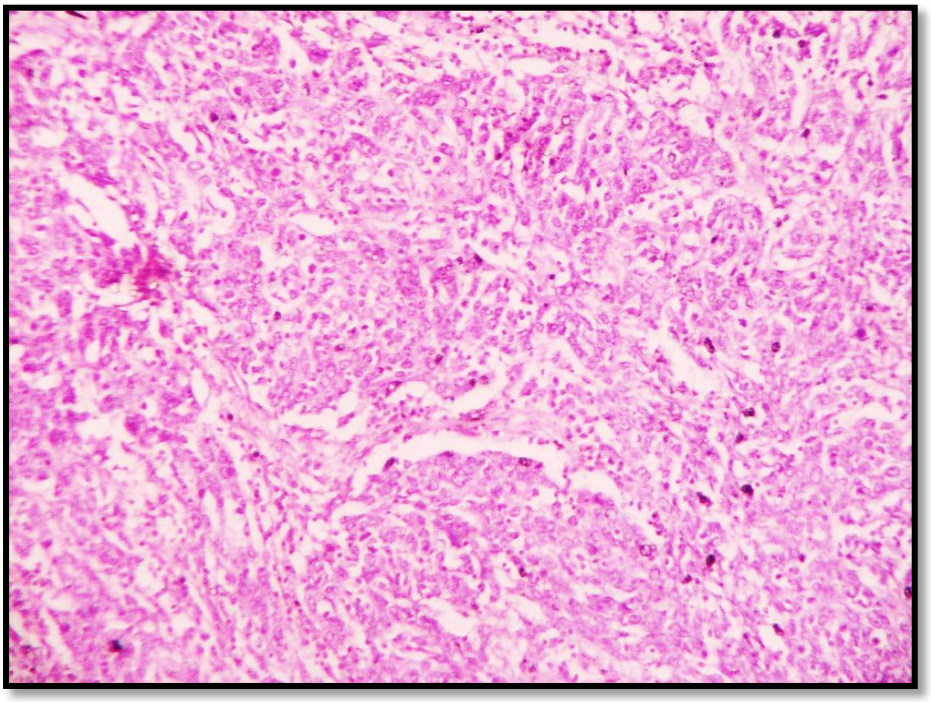


Fig.5: H & E, IDC grade 2, 200 X magnification

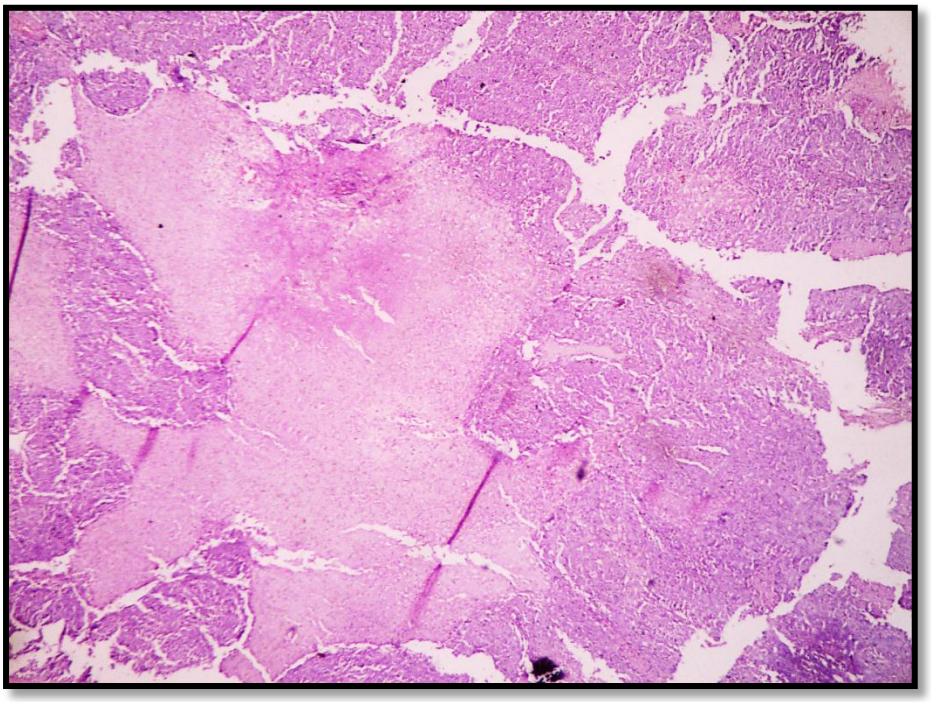


Fig.6: H & E, IDC grade 3, 200 X magnification

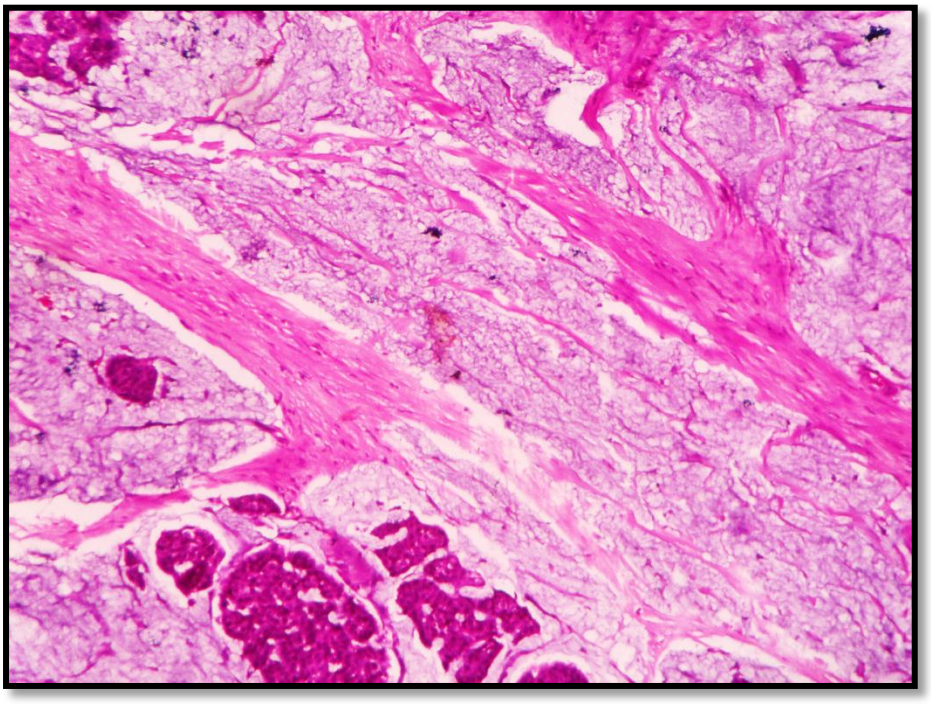


Fig.7: H & E : Colloid Carcinoma , 200 X magnification

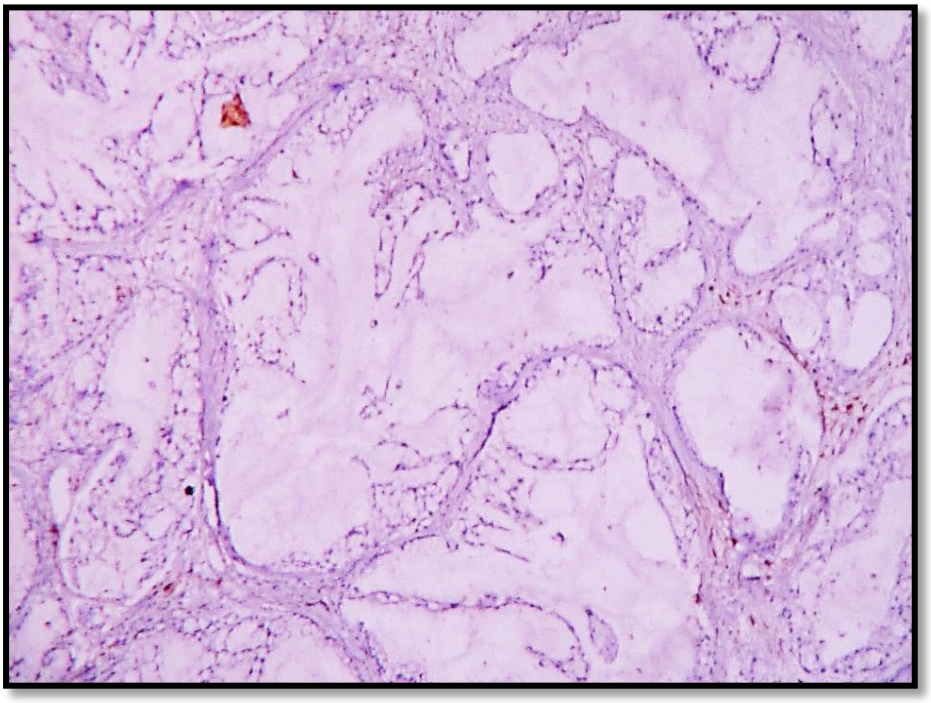


Fig.8: IHC , Colloid Carcinoma , 400 X magnification

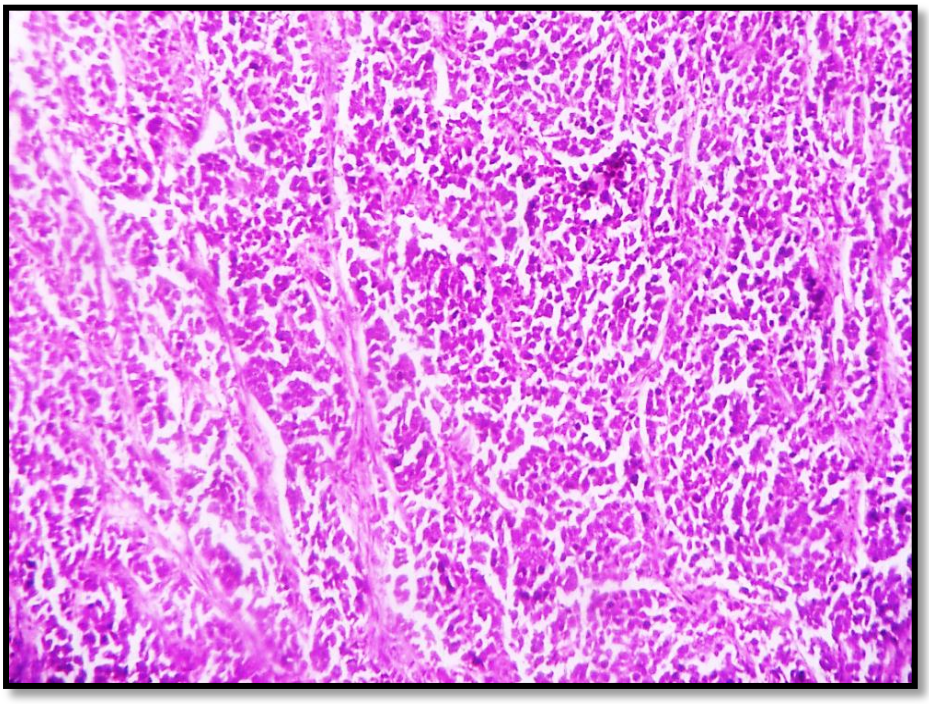


Fig.9: H & E, IDC with papillary differentiation, 200 X magnification

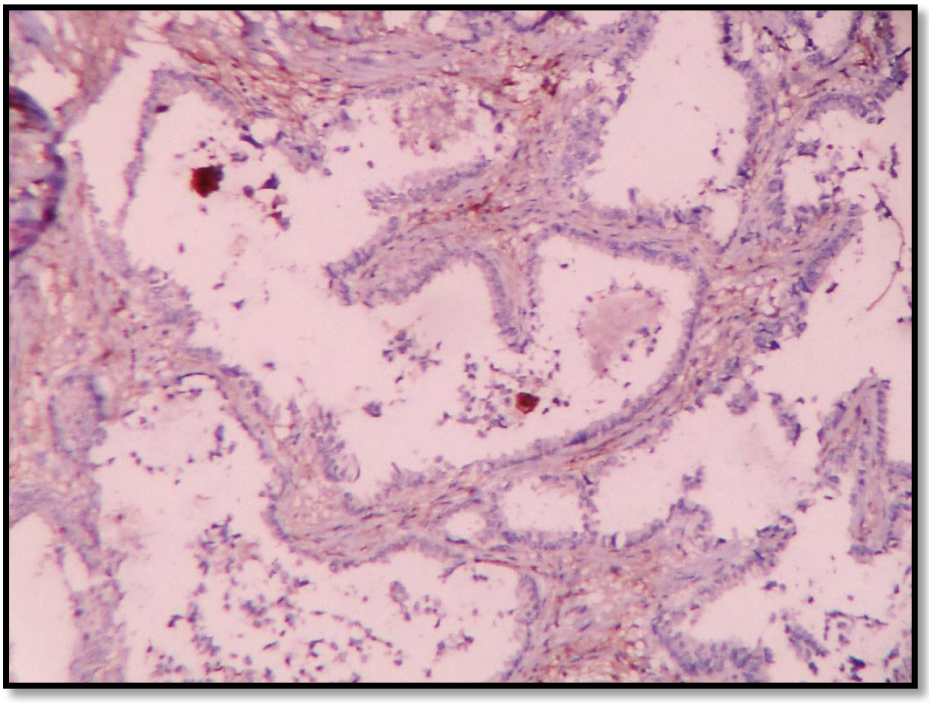


Fig.10: IHC CD 10 IDC with papillary differentiation,
Weak positive, 400 X magnification

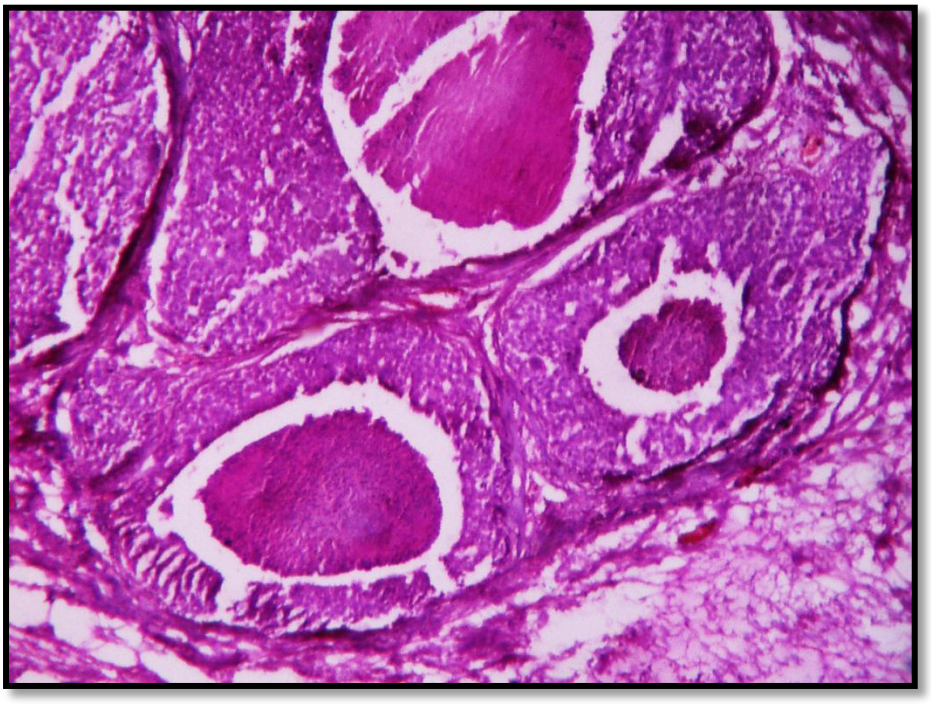


Fig.11: H and E, IDC with comedo necrosis,
Weak positive, 200 X magnification

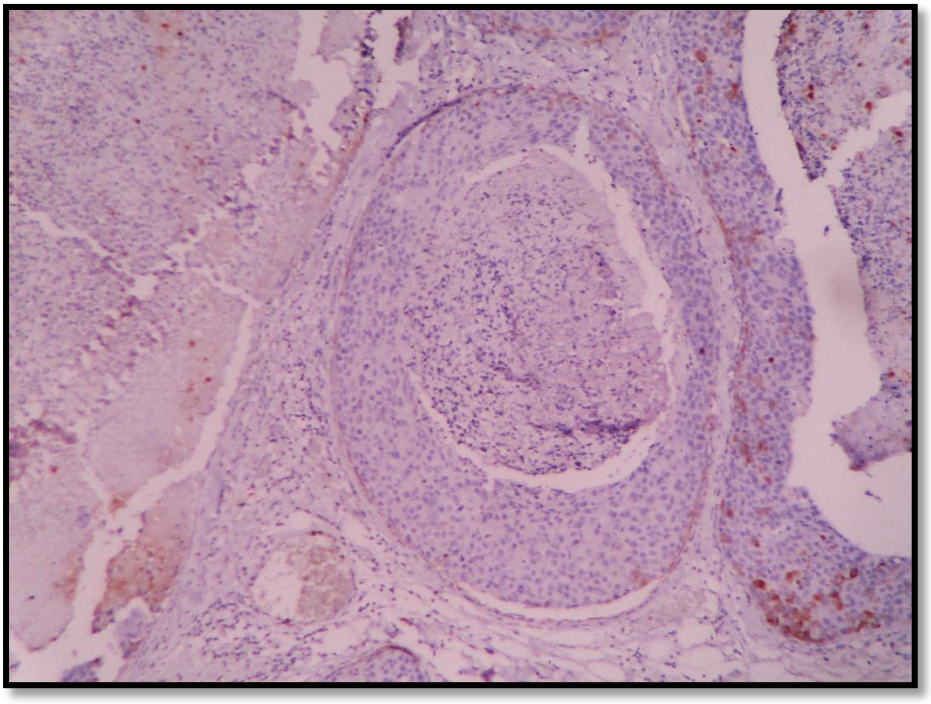


Fig.12: IHC CD 10 IDC with comedonecrosis,
200 X magnification

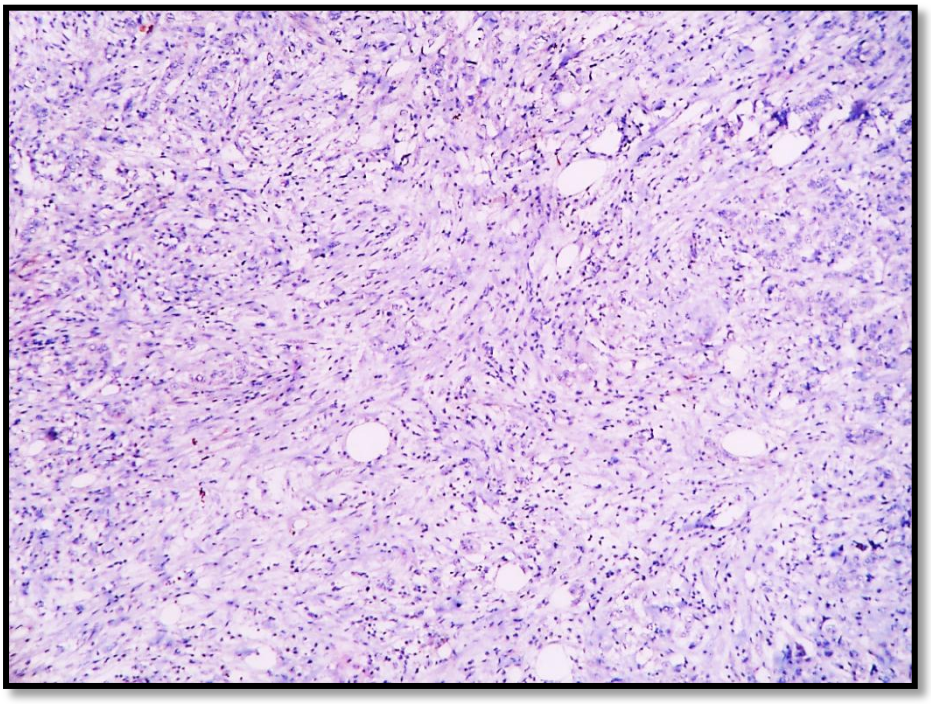


Fig.13: IHC CD10, Negative staining, 200 X magnification

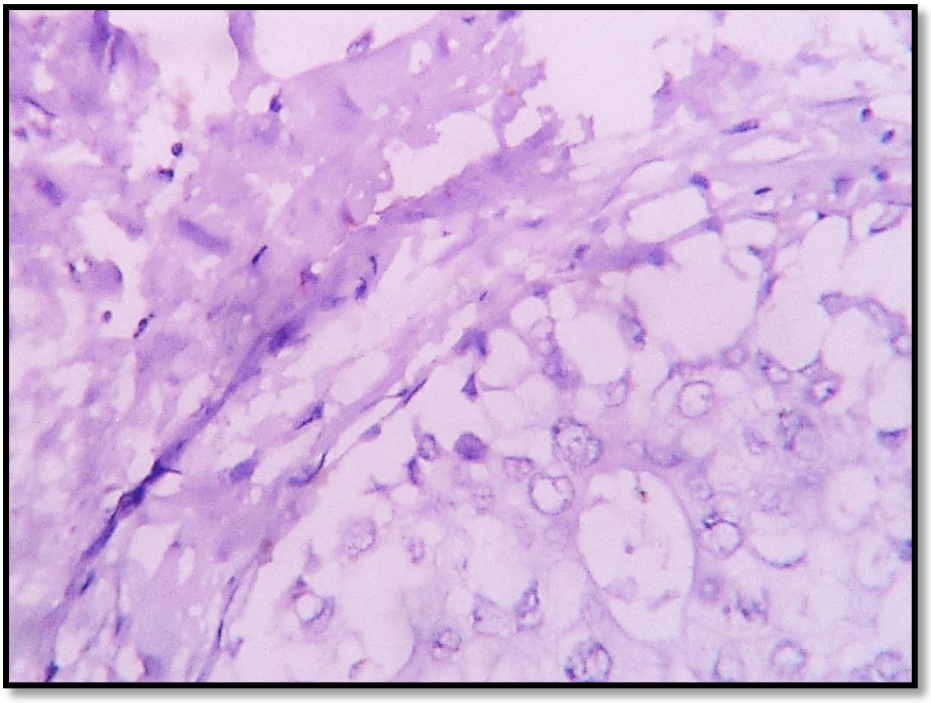


Fig.14: IHC CD10, Negative staining, 400 X magnification

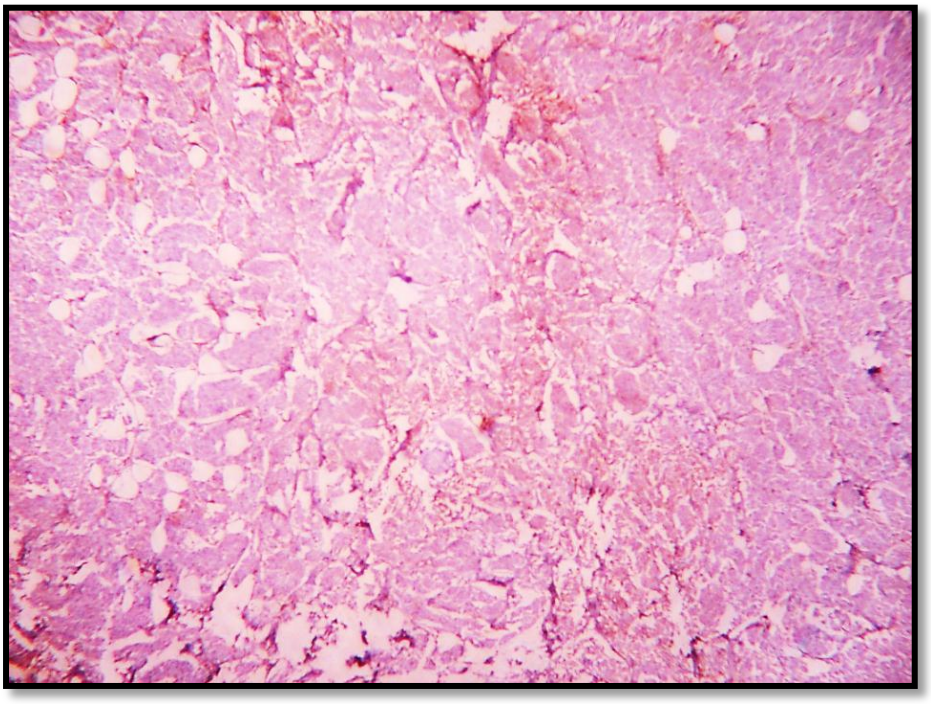


Fig.15: IHC CD10 Weak positive, 200 X magnification

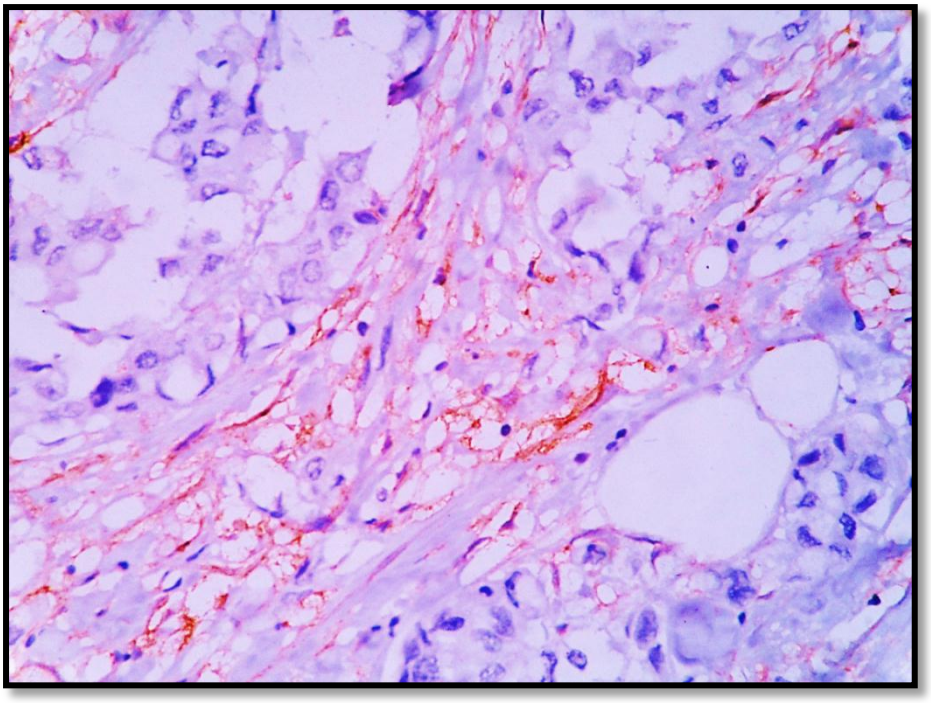


Fig.16: IHC CD 10, Weak positive, 400 X magnification

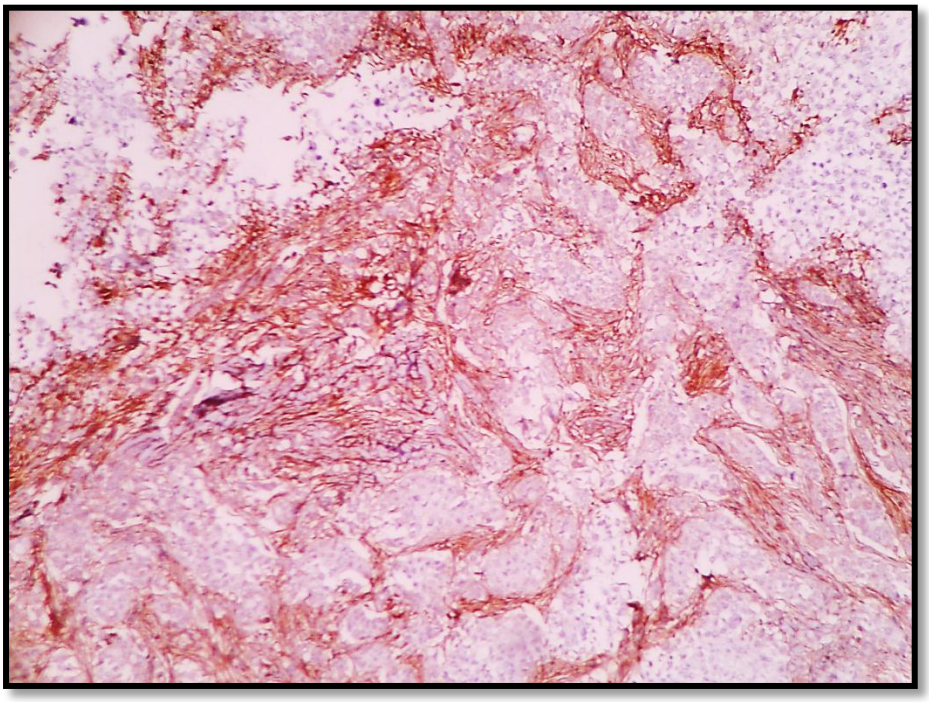


Fig.17: IHC CD 10, Strong positive, 200X magnification

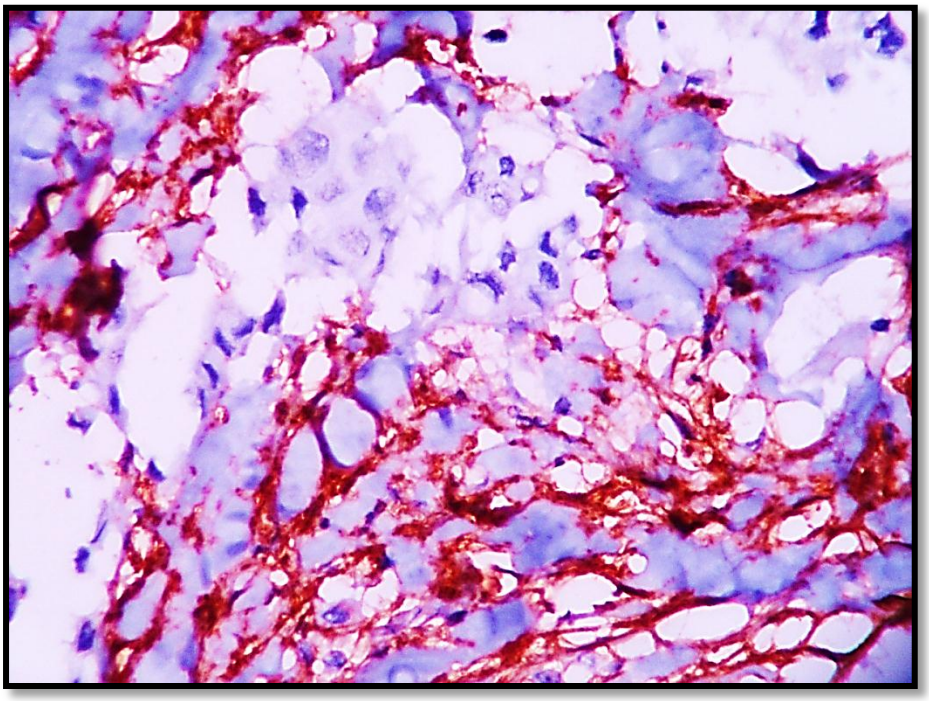


Fig.18: IHC CD 10, Strong positive, 400 X magnification

DISCUSSION

Age wise distribution of study sample

Our study had included 60 patients of breast carcinoma with age distribution from less than 30 years to more than 70 years with mean age of 48 years (48.86). Most of the patients in our sample population were of reproductive and premenopausal age group namely between 31 to 50 years.

A study conducted by Vandana puri et al in the year 2011 included patients from 30 to 80 years with a mean age of 48.5 years.

Sayantan H. Jana et al in the year 2014 conducted a study which included patients with age less than 40 to more than 60 years.

Comparison of CD 10 expression with age

Comparison of the CD10 expression with age, showed extremes of age showing weak positivity. The reproductive age group showed maximum association with positivity for CD10 expression. But it could not achieve statistical significance (P value 0.51). 29 out of 38 were positive (13 were weakly positive and 16 were strongly positive).

Keiichi Iwaya et al had 93 out of 110 patients belonging to <40 years and their comparison of CD10 expression with age did not show statistical significance (P value 0.95).

Fereshteh Mohammadizadeh et al (2012) in his study concluded that age parameter was not significantly associated with CD10 expression (p value 0.49).

Sayantan H Jana et al (2014) observed that comparison of age with CD10 positivity was not statistically significant (P value- 0.3572).

Ali Taghizadeh-Kermani et al(2014), found that 67 out of 100 cases of invasive carcinoma belonged to age group 41-60 years and comparison of age with CD10 positivity was not statistically significant (P value- 0.21).

B.V. Anuradha Devi et al (2016) had 26 out of 59 patients in the age group 41-60 years. Had found out no significance of expression of CD 10 with age parameter.

Table 20: Comparison of age wise distribution with CD 10 expression with other studies

Study	Age group	CD 10 expression	P value
Present study	31-50 years	29 out of 38 positive	0.51
Keiichi Iwaya et all	>40 years	17 out of 93 positive	0.95
Fereshteh Mohammadizadeh et al	-	-	0.49
Sayantan H. Jana et al	40 – 60 years	19 out of 45 positive	0.35
Ali Taghizadeh-Kermani et al	41-60 years	44 out of 67 positive	0.21
B.V. Anuradha Devi et al	41-60 years	12 out of 26 positive	0.52

Laterality

In the present study we had more or less an equal distribution with respect to laterality with 28 patients having left sided disease and 32 right sided disease. Though the number of weak and strong positivity differed in each, it could not attain statistical significance (P value 0.56).

Menopausal status

The present study has more number of post-menopausal patients. 34 out of 60, were post-menopausal of them 27 showed positivity with CD 10 expression (13 were weakly positive and 14 were strongly positive). No statistical correlation was achieved (P value 0.86).

Sayantan H. Jana et al had 42 out of 70 patients in the pre-menopausal age group and 27 out of 42 showed positive CD10 expression. Comparison of CD10 expression with menopausal status showed not statistical association (P value 0.47).

Ali Taghizadeh-Kermani et al had equal distribution of patients with 50 in each group. Though there was higher CD 10 positivity in post-menopausal group, it could not reach statistical significance (P value 0.28).

B.V. Anuradha Devi et al had 33 out of 59 patients in pre-menopausal age, with 19 out of 33 showing positive CD 10 expression. But no statistical association could be proved (P value 0.21).

All the studies showed no statistical association of CD 10 expression with menopausal status.

Table 21: Comparison of menopausal status with CD 10 expression with other studies

Study	Menopausal status	CD 10 expression	P value
Present study	Post-menopausal	27 out of 34 positive	0.86
Sayantan H. Jana et al	Pre-menopausal	22 out of 42 positive	0.47
Ali Taghizadeh-Kermani et al	Equal distribution 50 in each group	28 out of 50 positive Premenopausal 36 out of 50 positive Post-menopausal	0.28
B.V. Anuradha Devi et al	Pre-menopausal	19 out of 33 positive	0.21

Histologic subtype

In our study maximum number of patients were of IDC NST subtype namely 54 out of 60 cases. 44 out of the 54 IDC NST showed positive immunoreactivity with CD 10. The other subtypes were IDC with mucinous differentiation, IDC with comedo necrosis, IDC with Papillary differentiation. Though maximum positive immunoreactivity was seen with IDC NST, we could not prove a statistical significance (p value 0.52). Maybe due to sparse distribution of other subtypes.

Fereshteh Mohammadizadeh et al had 40 out of 44 patients with IDC NOS similar to our present study but could not get a statistical significance (P value 0.55).

Table 22: Comparison of histological subtype with CD 10 expression with other studies

Study	Maximum number of patients	CD10 expression	P value
Present study	IDC NST	44 out of 54 positive	0.52
Fereshteh Mohammadizadeh et al	IDC NOS(40)	-	0.55

Tumor size

Out of 60 patients in the study sample, 38 patients were having a tumor size 2 to 5 cm. 29 showed positive immunoreactivity with CD 10, but could not be prove a statistical relationship of CD 10 with tumor size. (P value 0.31).

Keiichi Iwaya et al study had 86 out 110 patients having a tumor size less than 5cm. 16 out the 86 showed positive CD10 expression. The comparison of CD10 expression with tumor size had not been statistically associated (P value 0.82).

Fereshteh Mohammadizadeh et al had done comparison of tumor size with CD10 expression and found the association to be statistical significant (P value 0.01).

Sayantan H. Jana et al study showed 30 out of 110 patients having tumor size between 2 to 5 cm. 15 out of the 30 showed positive for CD10 expression. The comparison of CD10 expression with tumor size showed no statistical significance (P value 0.53).

Ali Taghizadeh-Kermani et al study had 81 out of 110 patients with tumor size >2cm. 53 out of 81 had positive immunoreactivity with CD10 expression. The comparison of tumor size with CD10 expression statistically showed significant association (P value 0.04).

B.V. Anuradha Devi et al study showed 35 out of 59 patients having a tumor size between 2 to 5cm. 16 out of the 35 patients had positive CD10 expression. Comparison of CD10 expression with tumor size showed statistical significant association (P value 0.003).

Results similar to our study was noted in study done by Keiichi Iwaya et al and Sayantan H Jana et al.

Table 23: Comparison of tumor size with CD 10 expression with other studies

Study	Maximum number of patients with tumor size	CD 10 expression	P value
Present study	2- 5cm	29 out of 38 positive	0.31
Keiichi Iwaya	Less than 5 cm	16 out of 86 positive	0.82
Sayantan H. Jana et al	T 2(2-5cm)	15 out of 30 positive	0.53
Ali Taghizadeh-Kermani et al	>2cm	53 out of 81 positive	0.04
B.V. Anuradha Devi et al	2-5 cm	16 out of 25 positive	0.003

Mitotic rate

In our study, we had 21 patients with mitotic score 1, and 1 patient with grade 3. Maximum number of patients had score 2, namely 38 patients. 32 out of the 38 patients showed positive CD 10 expression, though this parameter showed no statistically significance (P value 0.44).

Sayantan H. Jana et al had 36 out of 70 patients having mitotic score 1 carcinoma out of whom 13 showed CD 10 positivity. The association was further proved with statistical significance (P value 0.03). This statistical significance was different from our study, maybe due to difference in mitotic counts observed in the study populations.

Table 24: Comparison of mitotic grade with CD 10 expression with other studies

Study	Maximum number of patients	CD10 expression	P value
Present study	Grade 2	32 out of 38 positive	0.44
Sayantan H. Jana et al	Grade 1	13 out of 36 positive	0.03

Lymph node status and grading [77,90]

In our study we had tried to analyse lymph node metastasis by evaluating total number of lymph nodes involved and also by lymph node staging of TNM. We had 17 patients in stage 3 and all 17 showed positive immunoreactivity with CD10 expression. Out of the 16 patients in stage 2, only 1 showed negative immunoreactivity. Maximum number of patients had no metastasis in lymph node, 27 out of 60. Of them, 15 showed positive CD10 expression. It was also seen that with increase in the number of lymph node metastasis, stronger the intensity of CD10 expression. This association was proved statistically (P value 0.01).

Keiichi Iwaya had 60 out of 110 patients with lymph node metastasis, of them 15 out of 60 showed positive CD10 immunoreactivity. The comparison of CD10 expression with lymph node metastasis showed statistical significance.

Fereshteh Mohammadizadeh et al study had 23 out of 49 patients with no lymph node metastasis. 16 out of the 23 patients had positive CD10 expression. On comparison of CD10 expression with lymph node metastasis, statistical significance was proved (0.02).

Sayantan H. Jana et al study had 30 out of 70 patients with a lymph node ratio <0.2. 13 out of the 30 patients had positive CD 10 expression. On comparison CD10 expression with lymph node metastasis showed no statistical significance (P value 0.18).

Ali Taghizadeh-Kermani et al study had 46 out of 100 patients with no lymph node metastasis. 20 out of the 46 showed positive CD10 immunoreactivity. The association was found to be statistically significant (P value <0.01).

B.V. Anuradha Devi et al study had 18 out of 59 patients with no lymph node metastasis. 10 out 18 had positive CD10 immunoreactivity. On statistical analysis the association was found to be significant (P value 0.0005).

All the studies showed significant association of CD10 expression with lymph node metastasis except Sayantan H Jana et al which may be due to difference in assessing lymph node involvement. They had used lymph node ratio instead of direct metastasis.

Table 25: Comparison of lymph node stage with CD 10 expression with other studies

Study	Maximum number of patients – lymph node status	CD10 expression	P value
Present study	No metastasis	15 out of 27 positive	0.01
Keiichi Iwaya	Metastasis	15 out of 60 positive	0.03
Fereshteh Mohammadizadeh et al	No metastasis	16 out of 23 positive	0.02
Sayantan H. Jana et al	Lymph node ratio <0.2	13 out 30 positive	0.18
Ali Taghizadeh-Kermani et al	No metastasis	20 out of 46 positive	<0.001
B.V. Anuradha Devi et al	No metastasis	10 out of 18 positive	0.0005

Histopathological grading

In our study, 47 out of the 60 patients (78%) were grade2. 37 out of 47 showed positive immunoreactivity with CD 10 expression. 4 patients belonged to grade 3 and all of them showed strong positive immunoreactivity with CD 10 expression. This shows that with increasing grade, the CD 10 expression increases potentially conveying it as a marker of aggressiveness of carcinoma. This relationship of histological grade as a parameter with the CD 10 expression revealed statistical significance. (P value 0.04).

Keiichi Iwaya et al had 47 out of 110 patients belonging to histopathological grade II, out of them 37 were positive for CD10 expression, but statistical analysis was not proved to be significant (P value 0.48).

Nikita A Makretsov et al, similar to the present study, had 139 put of 258 patients in histopathological grade II. 100 of whom showed positive CD10 expression. The comparison of CD10 with grade showed statistical association (P value 0.02).

Vandana Puri et al study had maximum grade 3 patients. The comparison of CD 10 expression with histopathologic grade did not turn out to be statistically significant (P value 0.13).

Fereshteh Mohammadizadeh et al had 25 out of 49 patients in grade 2 with 20 whom showed CD10 expression. The comparison was statistically proved to be significant (P value 0.004), similar to the present study.

Sayantan H. Jana et al study showed 28 out of 70 patients in histopathological grade 2 of them 16 showed CD10 positivity. Comparison of CD10 expression with tumor grade was associated significantly (P value 0.04), similar to the present study.

Ali Taghizadeh-Kermani et al had in their study 50 out of 100 patients belonging to tumor grade 2, with 31 out of 50 showing positive immunoreactivity with CD10 antigen. The comparison of CD10 expression

with tumor grade was statistical significant (P value <0.001), similar to the present study.

Hala N. Hosni et al in their study had 19 out 50 patients belonging to tumor grade 2 with 18 out of 19 cases showing positive CD10 expression. The comparison of CD10 expression with tumor grade was statistically significant (P value <0.05) similar to the present study.

B.V. Anuradha Devi et al had 32 out of 59 patients in histological grade II with 20 out of 32 showing positive CD10 expression. The association was statistical significant (P value 0.001).

Maha E Salama et al had 18 out of 36 patients in tumor grade 2 with all the 18 of them showing positive CD10 expression which showed statistical significance (P value <0.001).

Majority of studies except Keiichi Iwaya et al and Vandana Puri et al had significant association of histopathological grade with CD10 expression. The differences in 2 studies could be due to other factors.

Table 26: Comparison of histopathological grade with CD 10 expression in other studies

Study	Maximum number of patients	CD 10 expression	P value
Present study	Grade II	37 out of 47 positive	0.04
Keiichi Iwaya et al	Grade II	17 out of 84	0.48
Nikita A Makretsov et al	Grade II	100 out of 139 positive	0.002
Vandana Puri et al	Grade III		0.13
Fereshteh Mohammadizadeh et al	Grade II	20 out of 25 positive	0.004
Sayantan H. Jana et al	Grade II	16 out of 28 positive	0.04
Ali Taghizadeh-Kermani et al	Grade II	31 out of 50 positive	<0.001
Hala N. Hosni et al	Grade II	18 out of 19 positive	<0.05
B.V. Anuradha Devi et al	Grade II	20 out of 32 positive	0.0016
Maha E Salama et al	Grade II	18 out of 18 positive	<0.001

Nottingham's Prognostic index

36 out of the 60 patients belonged to moderate prognostic group. 25 out of the 36 showed positive immunoreactivity for CD 10 (17 were weakly positive and 9 were strongly positive for CD 10 expression). It was evident that 11 out of the 15 PPG showed strong reactivity for CD 10 and all in the VPG showed strong immunoreactivity thus proving that CD 10 expression correlated well with prognostic index thereby can be used as a prognostic indicator. This relationship of NPI with CD10 expression was proved with statistical significance (p value 0.009).

Sayantan H Jana et al study had similar distribution of patients in the study group with 32 MPG. Out of 32, 18 showed positive CD 10 immunoreactivity. The CD 10 correlation showed statistical significance with p value of 0.01.

B.V. Anuradha Devi et al study had 18 patients in the PPG group and out of them, 15 showed positive immunoreactivity for CD 10. They had a statistical significant value of 0.0023.

Thus all the studies point out that CD 10 expression correlated well with prognostic indicator thereby indirectly revealing that it can be used as a prognostic indicator.

Table 27: Comparison of NPI prognostic groups with CD 10 expression in other studies

Study	Maximum number of patients	Cd10 expression	P value
Present study	MPG	25 out of 36 positive	0.009
Sayantan H. Jana et al	MPG	18 out of 32 positive	0.01
B.V. Anuradha Devi et al	PPG	15 out of 18 positive	0.0023

SUMMARY

Breast carcinoma is the leading cause of mortality in under developed countries. It accounts for 70,000 deaths in India/ year (2012). The most important factor for mortality is metastasis which is limelight of research today. The primary objective of this study is to find out the CD 10 stromal expression in breast carcinoma and its association with Nottingham's prognostic index thereby relating it as a prognostic marker.

We had studied CD 10 stromal expression of 60 breast carcinoma patients who had undergone Modified radical mastectomy.

Overall our study had 38 patients (68%) in the age group 31-50 years, with 19 patients showing positive CD10 expression. Though positivity was higher in this age group, it was not statistical significant (P value 0.51).

Increase in the intensity of staining was noted with post-menopausal status but could not reach statistical significance (P value 0.89).

Our study had 54 patients belonging to IDC NST histological subtype of them, 44 showed positive CD10 immunoreactivity. The difference in the expression of various histological subtypes could not be proved statistically maybe due to sparse distribution of patients in the other subtypes (p value 0.52).

Analysis of lymph nodes based on number of nodes involved (P value 0.017) and also by lymph node staging (p value 0.013) in correlation with CD 10 expression also showed significant statistical association.

With regards to the tumor size our study had 38 patients with 2-5cm sized tumors. It was observed that with increasing size, increase in intensity of staining was proportional but significance could not be proved (P value 0.31).

Maximum number of patients were of mitotic score 2 and it showed strong positive immunoreactivity with CD10 expression. But this could not reach statistical significance with P value of 0.44.

Histologically, grade 2 had the maximum number of patients. They showed more positive reaction and with increasing grade, increase in intensity was noted. This association had a statistical significance with P value 0.04.

Finally out of the 60 patients we had most of them with NPI of Moderate Prognostic Group. Also increasing intensity was noted with Poor Prognostic Group and Very Poor Prognostic Group. This was proved statistically with P value of 0.009. Thus the role of CD 10 marker as a prognostic one is affirmed and can be used as a prognostic indicator.

CONCLUSION

The present study was undertaken at Department of pathology, Chengalpattu Medical College from June 2014 to June 2016. Our sample size included 60 patients of invasive ductal breast carcinoma who had undergone modified radical mastectomy.

The stromal expression of CD 10 showed statistically significant correlation with lymph node metastasis, histopathological grade and Nottingham's prognostic index.

No significant association could be established statistically for age, menopausal status, tumor size, histopathological subtype and mitotic rate.

Increasing intensity noted in association with few parameters like post-menopausal status, mitotic rate but could not show statistical significance.

Thus CD10 can be used as a prognostic marker in equivalence with NPI and lymph node staging.

Further studies are needed to establish the CD10 stromal expression in invasive ductal carcinoma of breast for predicting overall survival rates and disease free survival rates. Survival rates, post chemotherapy alterations and response to doxorubicin, its prodrugs and chemoresisitance needs to be elaborately viewed which can take the molecular understanding to higher levels and CD 10 can even emerge as a theranostic marker.

BIBLIOGRAPHY

1. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray, F. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer; 2013. Available from: http://globocan.iarc.fr

2. Bray F, Ren JS, Masuyer E, Ferlay J. Estimates of global cancer prevalence for 27 sites in the adult population in 2008. Int J Cancer. 2013 Mar 1; 132(5):113345. doi: 10.1002/ijc.27711. Epub 2012 Jul 26.

3. Martelli G,Pilotti S, Coopmans de Yoldi G, Viganotti G, Fariselli G, Lepera P, Moglia D.Diagnostic efficacy of physical examination, Mammography, fine needle aspiration cytology (triple test) in solid lumps. An analysis of 1708 consecutive cases. Tumori 1990, 76(5): 476-479

4. Johnson RH, Chien FL, Bleyer A. Incidence of Breast Cancer With Distant Involvement Among Women in the United States, 1976 to 2009. JAMA. 2013;309(8):800-805. doi:10.1001/jama.2013.776.

5. Fredholm H, Eaker S, Frisell J, Holmberg L, Fredriksson I, Lindman H (2009) Breast Cancer in Young Women: Poor Survival Despite Intensive Treatment. PLoS ONE 4(11): e7695. doi:10.1371/journal. pone.0007695.

6. Anders CK, Johnson R, Litton J, Phillips M, Bleyer A. Breast Cancer Before Age 40 Years. Seminars in oncology. 2009;36(3):237-249. doi:10.1053/j.seminoncol.2009.03.001.

7. Kesse-Adu R, Shousha S. Myoepithelial markers are expressed in atleast 29% of oestrogen receptor negative invasive breast carcinoma.Mod Pathol 2004;17:646-52.

8. Iwaya K, Ogawa H, Izumi M, Kuroda M, Mukai K. Stromal expression of CD10 in invasive breast carcinoma: A new predictor of clinical outcome. Virchows Arch. 2002;440(6):589–93.

9. Makretsov NA, Hayes M, Carter BA,Dabiri S, Gilks CB, Huntsman DG. Stromal CD 10 expression in invasive breast carcinoma correlates with poor prognosis, estrogen receptor negativity, and high grade. Mod Pathol. 2007; 20(1):84-9.

10. Harris JR, Lippman ME, Osborne CK, Morrow M. Diseases of the Breast.Wolters Kluwer Health; 2012. Pg 3-37.

11. Hamilton NJ, Boyd JD,Mossman HW. Human embryology. Cambridge Heffer 1968:428.

12. Cardiff RD, Wellings SR. the comparative pathology of human and mouse mammary glands. J Mammary Gland Biol Neoplasia 194;4:105.

13. Hughes ESR. Development of the mammary gland. Ann R Coll Surg Engl 1950;6:99.

14. Dawson EK. A histological study of the normal mamma in relation to tumour growth: I. Early Development to maturity. Edinb Med J 1934;41:653.

15. Moffat DF Going JJ. Three dimensional anatomy of complete duct system in human breast: pathological and developmental implications. J Clin Pathol 1996;49:48.

16. Sinnatamby CS. Last's Anatomy: Regional and Applied. Elsevier Health Sciences UK; 2011.pg70-71.

17. Bland KI, Copeland EM. The Breast: Comprehensive Management of Benign and Malignant Diseases. Saunders/Elsevier; 2009.

18. Skandalakis JE, Colborn GL. Skandalakis' Surgical Anatomy: The Embryologic and Anatomic Basis of Modern Surgery. PMP; 2004.

19. Skandalakis JE, Gray SW. Embryology for Surgeons. 2nd ed. Baltimore: Williams & Wilkins, 1994.

20. Morehead JR Anatomy and embryology of the breast. Clin Obstet Gynecol 25:353-357, 1982.

21. Spratt JS Jr, Donegan WL: Anatomy of the breast. In Donegan WL, Spratt JS Jr (eds): Cancer of the breast, 3rd ed. Philadelphia, WB Saunders, 1979.

22. Montagna W, Macpherson EA: Some neglected aspects of the anatomy of the human breast. J Invest Dermatol 63:10-16; 1974.

23. Tobon H, Salazar H: Ultrastructure of human mammary gland.I.Development of fetal gland throughout gestation. J Clin Endocrinol Metab 39:443-456, 1974.

24. Haagensen CD: Anatomy of the mammary glands. In Haagensen CD(ed): Disease of the breast, 3rd ed. Philadelphi, WB Saunders, 1986.

25. Smith DJ Jr et al : Breast volume and anthropomorphic measurements: Normal values. Plast Reconstr Surg 78:331-335,1986.

26. Cowie AT: Overview of mammary gland. J Invest Dermatol 63:2-9,1974.

27. Copeland EM, Bland KI: the breast. In Sabiston DC Jr(ed): Essentials of surgery. Philadelphia, WB, Saunders, 1987.

28. Sarhadi NS, Shaw-Dunn J, Soutar DS. Nerve supply of the breast with special reference to the nipple and areola: Sir Astley Cooper revisited. Clin Anat 10:283-288, 1997.

29. Cunningham L: The anatomy of the arteries and veins of the breast. J Surg Oncol 9:71-85,1977.

30. Sakki S: Angiography of the female breast. Ann Clin Res 6 (suppl 12): 1-47,1974.

31. Lineham DC, Hill ADK, Akhurst T, et al. Itradermal radioolloid and intraparenchymal blue dye injection optimize sentinel node identification in breast cancer patients. Ann Surg Oncol 1999;6:450.

32. Hultborn KA, Larsen LG, Raghnult I. The lymph drainage from the breast to the axillary and parasternal lymph nodes: studied with aid of colloidal Au198. Acta Radiol 1955;43-52.

33. Tanis PJ, Nieweg OE, Olmod RAV, et al. Anatomy and physiology of lymphatic drainage of the breast from the perspective of sentinel node biopsy. J Am Coll Surg 2001;192:399.

34. Nathanson SD, Nachna DL,Gilman D, et al. Pathways of lymphatic drainage from the breast. Ann Surg Oncol 2001;8:837.

35. Cody HS, Fey JV,Akhurst T, et al. Complementary of blue dye and isotope in sentinel node localization for breast cancer: univariate and multivariate analysis of 966 procedures. Ann Surg Oncol 2001;8:13.

36. Boolbol SK,Key JV, Borgen PI, et al. Intradermal isotope injection: a highly accurate method of lymphactic mapping in breast carcinoma. Ann Surg Oncol 2001;8:20.

37. Vargas HI, Tolmos J, Agbunag RV, et al. A validation trial of subdermal injection compared with intraparenchymal injection of sentinel lymph node biopsy in breast cancer. Am Surg 2002;68:87.

38. Giuliano AE, Dale PS, Turner RR, Morton DL, Evans SW, Krasne DL. Improved axillary staging of breast cancer with sentinel lymphadenectomy. Ann Surg 222:394-401, 1995.

39. Borgstein P, Meijer S. Historical perspective of lymphatic tumor spread and the emergence of the sentinel node concept. Eur J Surg Oncol 1998;24:85-95.

40. Albertini JJ, Lyman GH, Cox C, Yeatman T, Balducci L, Ku N, Shivers S, Berman C, Wells K, Rapaport D, Shons A, Horton J, Greenberg H, Nicosia S, Clark R, Cantor A, Reintgen DS. Lymphatic mapping and sentinel node biopsy in the patient with breast cancer. JAMA 276(22):1818-1822, 1996.

41. Ross MH, Pawlina W. Histology: A Text and Atlas : with Correlated Cell and Molecular Biology. Wolters Kluwer/Lippincott Williams & Wilkins Health; 2011

42. Moinfar F. Essentials of Diagnostic Breast Pathology: A Practical Approach . Springer Berlin Heidelberg; 2007.pg 2 and 3.

43. Mills, Stacey E. Histology for Pathologists, 3rd Edition Lippincott Williams & Wilkins 2007. Pg 57-71

44. Bocker W, Moll R, Poremba C, et al. Common adult stem cells in the human breast give rise to glandular and myoepithelial cell lineages: a new cell biological concept. Lab Invest 2002;82:737.

45. Bocker W, Bier B, Freytag G, et al. An immunohistochemical study of the breast using antibodies to basal and luminal keratins, alpha-smooth muscle actin, vimentin, collagen IV and laminin. Part I: Normal breast and benign proliferative lesions. Virchows Arch A Pathol Anat Histopathol 1992;421:315.

46. Mcgreevy J, Bland K. The breast. In O'Leary JP(ed): Physiologic basis of surgery. Baltimore, Williams & Wilkins, 1996.

47. Kaplan CR:Endocrinology of the breast. In Mitchell GW (ed): The female breast and its disorders. Baltimore, Williams & Wilkins, 1990.

48. Potter CS, Watson RJ, Williams GT, et al. The effect of age and menstrual cycle upon proliferative activity of the human breast.Br J Cancer 1988;58:163.

49. Going JJ, Anderson TJ, Battersby S, et al. Proliferative and secretory activity in human breast during natural and artificial menstrual cycles. Am J Pathol 1988;130:193.

50. Soderqvist G, Isakkson E, Schowltz BV, et al. Proliferation of breast epithelial cells in healthy women during menstrual cycle. Am J Obstet Gynecol 1997;176:123.

51. Fanager H, Ree HJ. Cyclic changes of human mammary gland epithelium in relation to the menstrual cycle-an ultrastructural study. Cancer 1974;34:574-585.

52. Huston SW, Cowen PN, Bird CC. Morphologic studies of age-related changes in normal human breast and their significance in the evolution of mammary cancer. J Clin Pathol 1985;38:281–287.

53. Monica Morrow, MD, Virgil Craig Jordan et al ,Managing Breast cancer Risk,illustrated,PMP-USA;2003:pg 47

54. Jaun Rosai MD. Rosai and Ackerman's Surgical pathology. Elsevier 2011. Pg 1659-1770.

55. Parkin DM, Bray FI, Devesa SS: Cancer burden in the year 2000. The global picture. *Eur J Cancer* 2001; 37:S4-66.

56. Armstrong K, Eisen A, Weber B: Assessing the risk of breast cancer. *N Engl J Med* 2000; 342:564-571

57. Moore DH, Moore II DH, Moore CT: Breast carcinoma etiological factors. *Adv Cancer Res* 1983; 40:189-253.

58. Kelsey JL, Gammon MD, John EM: Reproductive factors and breast cancer. *Epidemiol Rev* 1993; 15:36-47.

59. Wang DY, Rubens RD, Allen DS, Millis RR, Bulbrook RD, Chaudary MA, Hayward JL: Influence of reproductive history of age at diagnosis of breast cancer and prognosis. *Int J Cancer* 1985; 36:427-432

60. Clemons M, Goss P: Estrogen and the risk of breast cancer. *N Engl J Med* 2001; 344:276-285.

61. Bush TL, Whitemen M, Flaws JA. Hormone replacement therapy and breast cancer. a qualitative review. Obstet Gynecol 2001; 98:498-508.

62. Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women with breast cancer. Lancet 1997; 350: 1047-59.

63. Chen CL, Weiss NS, Newcomb P, Barlow W, White E: Hormone replacement therapy in relation to breast cancer. *JAMA* 2002; 287:734-741.

64. Ross RK, PaganiniHill A, Gerkins VR Mack TM, Pfeffer R, Arthur M, Henderson BE: A case-control study of menopausal estrogen therapy and breast cancer. *JAMA* 1980; 243:1635-1639.

65. Huang Z, Hankinson SE, Colditz GA, et al. Dual effects of weight and weight gain on breast cancer (United States). Cancer Causes Control 2000;11:533-42.

66. Friedenreich CM, Courneya KS, Bryant HE. Case-control study of anthropometric measures and breast cancer risk. Int J Cancer 2002;99:445-52.

67. Friedenreich CM, Thune I, Brinton LA, albanes D. Epidemiologic issues related to the association between physical activity and breast cancer. Cancer 1998; 83:600-610.

68. Skolnick MH, Cannon-Albright LA: Genetic predisposition to breast cancer. *Cancer* 1992; 70:1747-1754.

69. Anglian Breast Cancer Study Group : Prevalence and penetrance of BRCA1 and BRCA2 mutations in a population-based series of breast cancer cases. Anglian Breast Cancer Study Group. *Br J Cancer* 2000; 83:1301-1308.

70. Palacios J, Robles-Frias MJ, Castilla MA, Lopez-Garcia MA, Benitez J: The molecular pathology of hereditary breast cancer. *Pathobiology* 2008; 75:85-94.

71. Swift M, Morrell D, Massey RB, Chase CL: Incidence of cancer in 161 families affected by ataxia-telangiectasia. *N Engl J Med* 1991; 325:1831-1836.

72. Krieger N, Hiatt RA. Risk of breast cancer after benign breast diseases. Variation by histologic type, degree of atypia, age at biopsy, and length of follow-up. Am J Epidemiol 1992;135:619-31.

73. Hutchinson WB, Thomas DB, Hamlin WB, et al. Risk of breast cancer in women with benign breast disease. J Natl Cancer Inst 1980;65:13-20.

74. Wang J, Cosstantino JP, Tan-Chui E, et al. Benign breast disease and the risk of subsequent invasive breast cancer: findings from National Surgical Adjuvant Breast and Bowel Project's breast cancer prevention trial.Breast Cancer Res Treat. 2002;76:S36.

75. Fitzgibbons PL, Hensen DE, Hutter RV, et al. Benign breast changes and the risk for subsequent breast cancer.Arch Pathol Lab Med 1998;122:1053-5.

76. Goss PE, Sierra S: Current perspectives on radiation-induced breast cancer. *J Clin Oncol* 1998; 16:338-347.

77. Kurman RJ, cancer C international de recherche sur le, Carcangiu ML, Young RH, Herrington CS. WHO Classification of Tumours of Female Reproductive Organs. International Agency for Research on Cancer; 2014.

78. Fletcher SW, Elmore JG: Mammographic screening for breast cancer. *N Engl J Med* 2003; 348:1672-1680.

79. McLelland R: Screening mammography. *Cancer* 1991; 67:1129-1131.

80. Gonzalez JE, Caldwell RG, Valaitis J: Calcium oxalate crystals in the breast. Pathology and significance. *Am J Surg Pathol* 1991; 15:586-591.

81. American College of Radiology : Breast imaging reporting and data system (BI-RADS). ed. 3. Reston, VA, American College of Radiology, 1998.

82. Schnall MD: Breast MR imaging. *Radiol Clin North Am* 2003; 41:43-50. 83. Kneeshaw PJ, Turnbull LW, Drew PJ: Current applications and future direction of MR mammography. *Br J Cancer* 2003; 88:4-10.

84. Gisvold JJ: Imaging of the breast. Techniques and results. *Mayo Clin Proc* 1990; 65:56-66.

85. Arisio R, Cuccorese C, Accinelli G, Mano MP, Bordon R, Fessia L: Role of fine-needle aspiration biopsy in breast lesions: analysis of a series of 4,110 cases. *Diagn Cytopathol* 1998; 18:462-467.

86. Frable WJ: Needle aspiration of the breast. *Cancer* 1984; 53:671-676.

87. Laucirica R: Intraoperative assessment of the breast: guidelines and potential pitfalls. *Arch Pathol Lab Med* 2005; 129:1565-1574.

88. Sauter ER, Hoffman JP, Ottery FD, Kowalyshyn MJ, Litwin S, Eisenberg BL: Is frozen section analysis of reexcision lumpectomy margins worthwhile? Margin analysis in breast reexcisions. *Cancer* 1994; 73:2607-2612.

89. Esteban JM, Zaloudek C, Silverberg SG: Intraoperative diagnosis of breast lesions. Comparison of cytologic with frozen section technics. *Am J Clin Pathol* 1987; 88:681-688.

90. Christopher D.M. Flectcher. Diagnostic Histopathology of Tumours. Elsevier/Saunders.4th edition 2013.pg 1057-1145.

91. Rebecca H. Johnson, MD; Franklin L. Chien, BA; Archie Bleyer, MD. Incidence of Breast Cancer With Distant Involvement Among Women in the United States,1976 to 2009. *JAMA.* 2013; 309(8): 800805. doi:10.1001

92. Carey K. Anders, Rebecca Johnson, Jennifer Litton, Marianne Phillips, and Archie Bleyer. Breast Cancer Before Age 40 Years. *Semin Oncol*. 2009 June ; 36(3): 237–49 doi:10.1053

94. Reed W, Sandstad B, Holm R, Nesland JM: The prognostic impact of hormone receptors and c-erbB-2 in pregnancy-associated breast cancer and their correlation with BRCA1 and cell cycle modulators. *Int J Surg Pathol* 2003; 11:485-488.

95. Carter C L, Allen C, Henson D E 1989 Relation of tumour size,lymph node status,and survival in 24,270 breast cancer cases.Cancer 63: 181-187.

96. Beahrs O H, Shapiro S, Smart C et al. 1979 Summary report of the Working Group to review the National Cancer Institute–American Cancer Society Breast Cancer Demonstration Detectionprojects. J Natl Cancer Inst 62: 641-709

97. Rosen P P, Groshen S 1990 Factors influencing survival and prognosis in early breast carcinoma (T1N0M0-T1N1N0). Assessment of 644 patients with median follow-up of 18 years. Surg Clin North Am 70: 937-962

98. Tabar L, Duffy S W, Krusemo V B 1987 Detection method tumour size and node metastases in breast cancers diagnosed during a trial of breast cancer screening. Eur J Cancer Clin Oncol 23: 959-962

99. Tsakraklides V, Olson P, Kersey JH, Good RA: Prognostic significance of the regional lymph node histology in cancer of the breast. *Cancer* 1974; 34:1259-1266.

100. Ferguson D J, Meier P, Karrison T et al. 1982 Staging of breast cancer and survival rates: an assessment based on 50 years of experience with radical mastectomy. JAMA 248: 1337-1341

101. Schottenfeld D, Nash A G, Robbins G F et al. 1976 Ten year results of the treatment of primary operable breast carcinoma. Cancer 38: 1001-1007

102. Locker A P, Ellis I O, Morgan D A et al. 1989 Factors influencing local recurrence after excision and radiotherapy for primary breast cancer. Br J Surg 76: 890-894

103. Krag D N, Anderson S J, Julian T B et al. 2010 Sentinel-lymphnode resection compared with conventional axillary-lymph-node dissection in clinically node-negative patients with breast cancer:overall survival findings from the NSABP B-32 randomised phase 3 trial. Lancet Oncol 11: 927-933

104. Maaskant-Braat A J, van de Poll-Franse L V, Voogd A C et al.2011 Sentinel node micrometastases in breast cancer do not affect prognosis: a population-based study. Breast Cancer Res Treat 127:195-203

105. Ozao-Choy J, Giuliano A E 2011 Prognostic significance of micrometastasis and isolated tumor cells in the sentinel lymph node. Breast Cancer Res Treat 127: 205-206

106. Cascinelli N, Greco M, Bufalino R et al. 1987 Prognosis of breast cancer with axillary node metastases after surgical treatment only. Eur J Cancer Clin Oncol 23: 795-799

107. Donegan W L, Stine S B, Samter T G 1993 Implications of extracapsular nodal metastases for treatment and prognosis of breast cancer. Cancer 72: 778-782

108. Smith III JA, Gamez Araujo J, Gallager HS, White EC, McBride CM: Carcinoma of the breast. Analysis of total lymph node involvement versus level of metastasis. *Cancer* 1977; 39:527-532.

109. Haybittle J L, Blamey R W, Elston C W et al. 1982 A prognostic index in primary breast cancer. Br J Cancer 45: 361-366

110. Todd J H, Dowle C, Williams M R et al. 1987 Confirmation of a prognostic index in primary breast cancer. Br J Cancer 56:489-492

111. Galea M H, Blamey R W, Elston C E et al. 1992 The Nottingham Prognostic Index in primary breast cancer. Br Cancer Res Treat 22: 207-219

112. Veronique maguer-satta ,Roger besancon, Elodie bachelard-cascales. Concise review: neutral endopeptidase (cd10): a multifaceted environment actor in stem cells, physiological mechanisms,and cancer. Stem Cells Express January 7, 2011 ;29:389–396

113. Vandana Puri, Manjula Jain and Shaji Thomas. Stromal Expression of CD10 in Invasive Breast Carcinoma and Its Correlation with ER, PR, HER2neu and Ki67. International Journal of Breast Cancer. Volume 2011, Article ID 437957, 4 pages, doi:10.4061/2011/437957

114. Fereshteh Mohammadizadeh, Majid Salavati, Noushin Afshar Moghaddam. CD10 expression in stromal component of invasive breast carcinoma: A potential prognostic determinant. J Res Med Sci 2012;17 S194-9.

115. Hala N Hosni, Ah,med Abd El Aziz, Sahar A Tabak, Maha Elsayed. Immunohistochemical study of stromal CD 10 expression in mammary duct carcinoma.Med J Cairo., 2012 ;Vol 80, No2, June :37-44.

116. Sayantan H. Jana, Bharti M. Jha, Chandni Patel, Dipan Jana, Anshul Agarwal. CD10- A new prognostic stromal marker in breast carcinoma, its utility, limitations and role in breast cancer pathogenesis. Indian Journal of Pathology and Microbiology – 57(4), October-December 2014, DOI: 10.4103/0377-4929.142639

117. Ali Taghizadeh-Kermani et al, The stromal overexpression of CD10 in Invasive Breast Cancer and its Association with Clinicopathologic factors. Iran J Cancer Prev. 2014; 1:17-21

118. Maha E. Salama, Wael S. Ibrahim and Dina A.Khairy. Comparison of CD10 expression in stroma of epithelial and mesenchymal tumors of the breast. Global Advanced Research Journal of Medicine and Medical Science (ISSN: 2315-5159) Vol. 4(1) pp. 051-056, January, 2015

119. B.V. Anuradha Devi S, Chandra Sekhar C, Saritha S, Sanhya Anil H, Sandhya Rani. A study on stromal CD10 expression in invasive breast carcinoma. IAIM, 2016; 3(6):142-147.

120. Thomas S, Babu RJ, Agarwal K, Puri V, Jain M, Andley M, *et al*. Effect of neoadjuvant chemotherapy on stromal CD10 antigens in breast cancer - A preliminary study. Indian J Cancer 2013;50:46-51

ANNEXURE I

WHO HISTOLOGICAL CLASSIFICATION OF THE TUMORS OF BREAST

Epithelial tumours

- Invasive ductal carcinoma, not otherwise specified (NOS) - 8500/3
  - Mixed type carcinoma
  - Pleomorphic carcinoma - 8022/3
  - Carcinoma with osteoclastic giant cells - 8035/3
  - Carcinoma with choriocarcinomatous features
  - Carcinoma with melanotic features
- Invasive lobular carcinoma - 8520/3
- Tubular carcinoma - 8211/3
- Invasive cribriform carcinoma - 8201/3
- Medullary carcinoma - 8510/3
- Mucinous carcinoma & other tumours with abundant mucin
  - Mucinous carcinoma - 8480/3
  - Cystadenocarcinoma & columnar cell mucinous carcinoma - 8480/3
  - Signet ring cell carcinoma - 8490/3
- Neuroendocrine tumours
  - Solid neuroendocrine carcinoma
  - Atypical carcinoid tumour - 8249/3
  - Small cell / oat cell carcinoma - 8041/3
  - Large cell neuroendocrine carcinoma - 8013/3

- Invasive papillary carcinoma - 8503/3
- Invasive micropapillary carcinoma - 8507/3
- Apocrine carcinoma - 8401/3
- Metaplastic carcinomas - 8575/3
  - Pure epithelial metaplastic carcinomas - 8575/3
  - Squamous cell carcinoma - 8070/3
  - Adenocarcinoma with spindle cell metaplasia - 8572/3
  - Adenosquamous carcinoma - 8560/3
  - Mucoepidermoid carcinoma - 8430/3
  - Mixed epithelial/mesenchymal metaplastic carcinomas - 8575/3
- Lipid-rich carcinoma - 8314/3
- Secretory carcinoma - 8502/3
- Oncocytic carcinoma - 8290/3
- Adenoid cystic carcinoma - 8200/3
- Acinic cell carcinoma - 8550/3
- Glycogen-rich clear cell carcinoma - 8315/3
- Sebaceous carcinoma - 8410/3
- Inflammatory carcinoma - 8530/3
- Lobular neoplasia
  - Lobular carcinoma in situ - 8520/2
- Intraductal proliferative lesions
  - Usual ductal hyperplasia
  - Flat epithelial atypia
  - Atypical ductal hyperplasia
  - Ductal carcinoma in situ - 8500/2

- Microinvasive carcinoma
- Intraductal papillary neoplasms
  - Central papilloma - 8503/0
  - Peripheral papilloma - 8503/0
  - Atypical papilloma
  - Intraductal papillary carcinoma - 8503/2
  - Intracystic papillary carcinoma - 8504/2
- Benign epithelial proliferations
  - Adenosis including variants: sclerosing adenosis, apocrine adenosis, blunt duct adenosis, microglandular adenosis, adenomyoepithelial adenosis
  - Radial scar / complex sclerosing lesion
  - Adenomas
  - Tubular adenoma - 8211/0
  - Lactating adenoma - 8204/0
  - Apocrine adenoma - 8401/0
  - Pleomorphic adenoma - 8940/0
  - Ductal adenoma - 8503/0

Myoepithelial lesions

- Myoepitheliosis
- Adenomyoepithelial adenosis
- Adenomyoepithelioma - 8983/0
- Malignant myoepithelioma - 8982/3

Mesenchymal Tumors

- Hemangioma - 9120/0
- Angiomatosis
- Haemangiopericytoma - 9150/1
- Pseudoangiomatous stromal hyperplasia
- Myofibroblastoma - 8825/0
- Fibromatosis (aggressive) - 8821/1
- Inflammatory myofibroblastic tumour - 8825/1
- Lipoma - 8850/0
    - Angiolipoma - 8861/0
- Granular cell tumour - 9580/0
- Neurofibroma - 9540/0
- Schwannoma - 9560/0
- Angiosarcoma - 9120/3
- Liposarcoma - 8850/3
- Rhabdomyosarcoma - 8900/3
- Osteosarcoma - 9180/3
- Leiomyoma - 8890/0
- Leiomyosarcoma - 8890/3

Fibroepithelial Tumors

- Fibroadenoma - 9010/0
- Phyllodes tumour - 9020/1
    - Benign - 9020/0

    - Borderline - 9020/1
    - Malignant - 9020/3
- Periductal stromal sarcoma, low grade - 9020/3
- Mammary hamartoma

Tumors of the nipple

- Nipple adenoma - 8506/0
- Syringomatous adenoma - 8407/0
- Paget disease of the nipple - 8540/3

Malignant lymphoma

- Diffuse large B cell lymphoma - 9680/3
- Burkitt lymphoma - 9687/3
- Extranodal marginal-zone B-cell lymphoma of MALT type - 9699/3
- Follicular lymphoma - 9690/3

Metastatic tumors

Tumors of the male breast

- Gynaecomastia
- Carcinoma
    - Invasive - 8500/3
    - In situ - 8500/2

ANNEXURE II

DATA ENTRY FORM

Name:

Age: Sex : Male/Female

Diagnosis:

Nature of specimen: trucut

Modified radical mastectomy

Others (specify)

Laterality:

Size of tumour:

Histopathological diagnosis:

Histopathological grading:

No of nodes dissected:

No of nodes with metastasis:

Nottingham s prognostic index

CD expression:

Negative

Weak positive

Strong positive

Remarks (if any)

ANNEXURE III

MASTER CHART

S No.	HPE No	Age	Side	Diagnosis	Menopausal Status	HPE Grade	Number of Positive LN	Tumour Size (cm)	Mitotic rate	LN staging	NPI	IHC interpretation
1	g 71/14	46	left	IDC	Post	Grade 2	0	7	1	1	4.4	Negative
2	g 82/14	38	Right	IDC/ comedo	Pre	Grade 2	0	5	1	1	4	Negative
3	g 207/14	40	left	colloid	Pre	Grade 1	0	4.5	1	1	2.9	Negative
4	g 222/14	50	Right	IDC	Post	Grade 2	0	4	2	1	3.8	Weak Positive
5	g 302/14	70	right	IDC	Post	Grade 2	0	4	2	1	3.8	Weak Positive
6	G829/14	32	Left	IDC	Pre	Grade 2	1	3	2	2	4.6	Strong Positive
7	G 846/14	50	Right	IDC	Pre	Grade 2	0	5	1	1	4	Strong Positive
8	G 978/14	45	Right	IDC	Pre	Grade 2	17	4.8	1	3	5.96	Weak Positive
9	g1119/14	59	Right	IDC	Post	Grade 2	0	2.5	1	1	3.5	Weak Positive
10	g 1121/14	70	Right	IDC	Post	Grade 2	0	3	1	1	3.6	Negative
11	g 1184/14	65	left	IDC	Post	Grade 2	0	2	1	1	3.4	Strong Positive
12	g1203/14	58	left	IDC	Post	Grade 3	2	2.8	2	2	5.56	Strong Positive
13	g 1207 /14	45	left	IDC	Pre	Grade 2	1	2	1	2	4.4	Weak Positive
14	g1220/14	70	Left	IDC	Post	Grade 2	5	2.5	2	3	5.5	Weak Positive

S No.	HPE No	Age	Side	Diagnosis	Menopausal Status	HPE Grade	Number of Positive LN	Tumour Size (cm)	Mitotic rate	LN staging	NPI	IHC interpretation
15	g 1257/14	46	Right	IDC	Pre	Grade 1	2	10	2	2	5	Weak Positive
16	g 1315/14	50	Left	IDC	Post	Grade 2	1	7.5	2	2	5.5	Strong Positive
17	g 1317/14	40	Right	IDC	Pre	Grade 2	2	3.5	2	2	4.7	Strong Positive
18	g 1345/14	52	Left	IDC	Post	Grade 2	0	3	2	1	3.6	Negative
19	g 1352/14	47	Left	IDC	Post	Grade 2	0	2.5	3	1	3.5	Negative
20	g 1469/14	60	left	IDC	Post	Grade 2	11	3.7	2	3	5.74	Strong Positive
21	g 1491/14	45	Right	IDC	Pre	Grade 3	0	5	1	1	5	Strong Positive
22	g1503/14	56	left	IDC	Post	Grade 2	0	10	2	1	5	Negative
23	g 1517/14	48	left	IDC	Post	Grade 2	10	8	2	3	6.6	Strong Positive
24	g 1548/14	60	Right	IDC	Post	Grade 1	16	5	2	3	5	Weak Positive
25	g 1566/14	46	Right	IDC	Post	Grade 2	0	2	2	1	3.4	Weak Positive
26	g 1593/14	45	Right	IDC	Post	Grade 2	1	4	2	2	4.8	Negative
27	g 1678 /14	60	Right	IDC	Post	Grade 2	3	6.5	2	2	5.3	Strong Positive
28	G 3/15	35	left	IDC	Pre	Grade 2	0	13	2	1	5.6	Strong Positive
29	g 128/15	53	Right	IDC/Mucin	Post	Grade 2	0	6	1	1	4.2	Negative
30	g 148/15	40	left	IDC	Pre	Grade 2	7	4	1	3	5.8	Strong Positive
31	g 275/15	50	Right	IDC	Post	Grade 3	1	2.5	2	2	5.5	Strong Positive

S No.	HPE No	Age	Side	Diagnosis	Menopausal Status	HPE Grade	Number of Positive LN	Tumour Size (cm)	Mitotic rate	LN staging	NPI	IHC interpretation
32	g 433/15	39	Right	IDC	Pre	Grade 2	0	4.5	2	1	3.9	Negative
33	g 460/15	78	Right	IDC	Post	Grade 2	3	4	1	2	4.8	Weak Positive
34	g 482/15	35	left	IDC	Pre	Grade 2	3	2	2	2	4.4	Weak Positive
35	g 497/15	25	left	IDC	Pre	Grade 2	0	1.3	1	1	3.26	Weak Positive
36	g647/15	60	Right	IDC	Post	Grade 2	12	7.5	2	3	6.5	Strong Positive
37	g945/15	38	Left	IDC	Pre	Grade 1	0	2	1	1	2.4	Negative
38	g 1027/15	65	Right	IDC	Post	Grade 2	8	3	2	3	5.6	Weak Positive
39	g 1075/15	60	Right	IDC	Post	Grade 2	3	3	2	2	4.6	Weak Positive
40	g 1304/15	50	left	IDC/Mucin	Post	Grade 2	11	3.5	2	3	5.7	Strong Positive
41	g 1416/15	39	Right	IDC	Pre	Grade 1	8	2	1	3	4.4	Strong Positive
42	g 1450/15	35	left	IDC	Pre	Grade 1	8	2	1	3	4.4	Weak Positive
43	g 1521/15	70	left	IDC	Post	Grade 2	0	3.5	2	1	3.7	Strong Positive
44	g 1562/15	42	left	IDC	Pre	Grade 2	0	5	2	1	4	Weak Positive
45	g 1565/15	44	left	IDC	Post	Grade 2	7	3	2	3	5.6	Strong Positive
46	g 1600/15	53	left	IDC	Post	Grade 2	8	6	2	3	6.2	Weak Positive
47	g 1686/15	50	Right	IDC	Post	Grade 2	8	2.5	2	3	5.5	Strong Positive
48	g 1696/15	38	Right	IDC	Pre	Grade 2	3	3.5	1	2	4.7	Weak Positive

S No.	HPE No	Age	Side	Diagnosis	Menopausal Status	HPE Grade	Number of Positive LN	Tumour Size (cm)	Mitotic rate	LN staging	NPI	IHC interpretation
49	g 125/16	40	Right	IDC	Pre	Grade 2	0	7	2	1	4.4	Weak Positive
50	g275/16	35	Right	IDC /pap	Pre	Grade 1	0	2.8	1	1	2.56	Weak Positive
51	g 492/16	62	Right	IDC	Post	Grade 2	1	2.5	2	2	4.5	Strong Positive
52	g 514/16	56	left	IDC	Post	Grade 2	1	4	2	2	4.8	Weak Positive
53	g 579/16	48	Right	IDC	Post	Grade 2	7	7	2	3	6.4	Strong Positive
54	g 642/16	36	left	IDC	Pre	Grade 2	0	3.5	2	1	3.7	Negative
55	g 701 /16	45	left	IDC	Pre	Grade 2	2	1.5	1	2	4.3	Weak Positive
56	g 796/ 16	43	Right	IDC	Pre	Grade 3	0	2.5	2	1	4.5	Strong Positive
57	g 876/16	40	Right	IDC	Pre	Grade 1	0	4.5	2	1	2.9	Negative
58	g 905/16	69	Right	IDC/Mucin	Post	Grade 2	0	4	2	1	3.8	Weak Positive
59	g 1006/16	50	Right	IDC	Post	Grade 1	4	2	2	3	4.4	Weak Positive
60	g1087/16	40	Left	IDC	Pre	Grade 2	17	5	1	3	6	Strong Positive

Abbreviations in master chart:

HPE no	:	Histopathology number
Age	:	Age in years
Side	:	Laterality, Side of diseased breast
IDC	:	Invasive Ductal Carcinoma No Specific Type
IDC/comedo	:	Invasive Ductal Carcinoma with comedo necrosis
IDC/mucin	:	Invasive Ductal Carcinoma with mucinous areas
IDC/Pap	:	Invasive Ductal Carcinoma with papillary areas
Colloid	:	Colloid carcinoma
Pre	:	Pre-menopausal
Post	:	Post-menopausal
HPE Grade	:	Elston-Ellis modification of Scarff Bloom Richardson Histopathological Grade.
Positive LN	:	Total number of positive lymph nodes
Tumour size	:	greatest dimension of tumour
Mitotic rate	:	Mitotic rate assessed by Elston-Ellis modification of Scarff Bloom Richardson Histopathological Grade.
LN stage	:	Lymph node staging by AJCC/TNM classification
NPI	:	Nottingham's Prognostic index
IHC		
Interpretation	:	Immunohistochemical interpretation of stromal expression of CD10 antigen.

ANNEXURE IV: GLOSSARY

BIRADS	:	Breast Imaging Reporting And Data System
BRCA1	:	Breast cancer 1
CD	:	Cluster of Differentiation
DAB	:	Diaminobenzidine
DCIS	:	Ductal carcinoma in situ
ER	:	Estrogen receptor
FSH	:	Follicle stimulating hormone
LH	:	Luteinizing hormone
GnRH	:	Gonadotropin releasing hormone
HER2/neu	:	Human epidermal growth factor receptor 2
Hpf	:	High power field
HRP	:	Horse radish peroxidase
IHC	:	Immunohistochemistry
MiB1	:	Mindbomb 1
PIP3	:	Phosphatidylinositol trisphosphate
PTEN	:	Phosphatase and tensin homolog
PR	:	Progesterone receptor
SHBG	:	Sex hormone binding globulin
IDC	:	Invasive Ductal Carcinoma
IDC NST	:	Invasive Ductal Carcinoma- No specific Type
TNM	:	Tumour node metastasis

TRIS	:	tris-(hydroxymethyl) aminomethane
EPG	:	Excellent Prognostic Group
GPG	:	Good Prognostic Group
MPG	:	Moderate Prognostic Group
PPG	:	Poor Prognostic Group
VPG	:	Very Poor Prognostic Group
MMP	:	Matrix metalloproteases
FAK	:	Focal adhesion kinase
FGF	:	Fibroblast growth factor
PI3K	:	Phosphatidylinositol 3-kinases
PIP3	:	Phosphatidylinositol 3,4,5-trisphosphate